Fig 1A

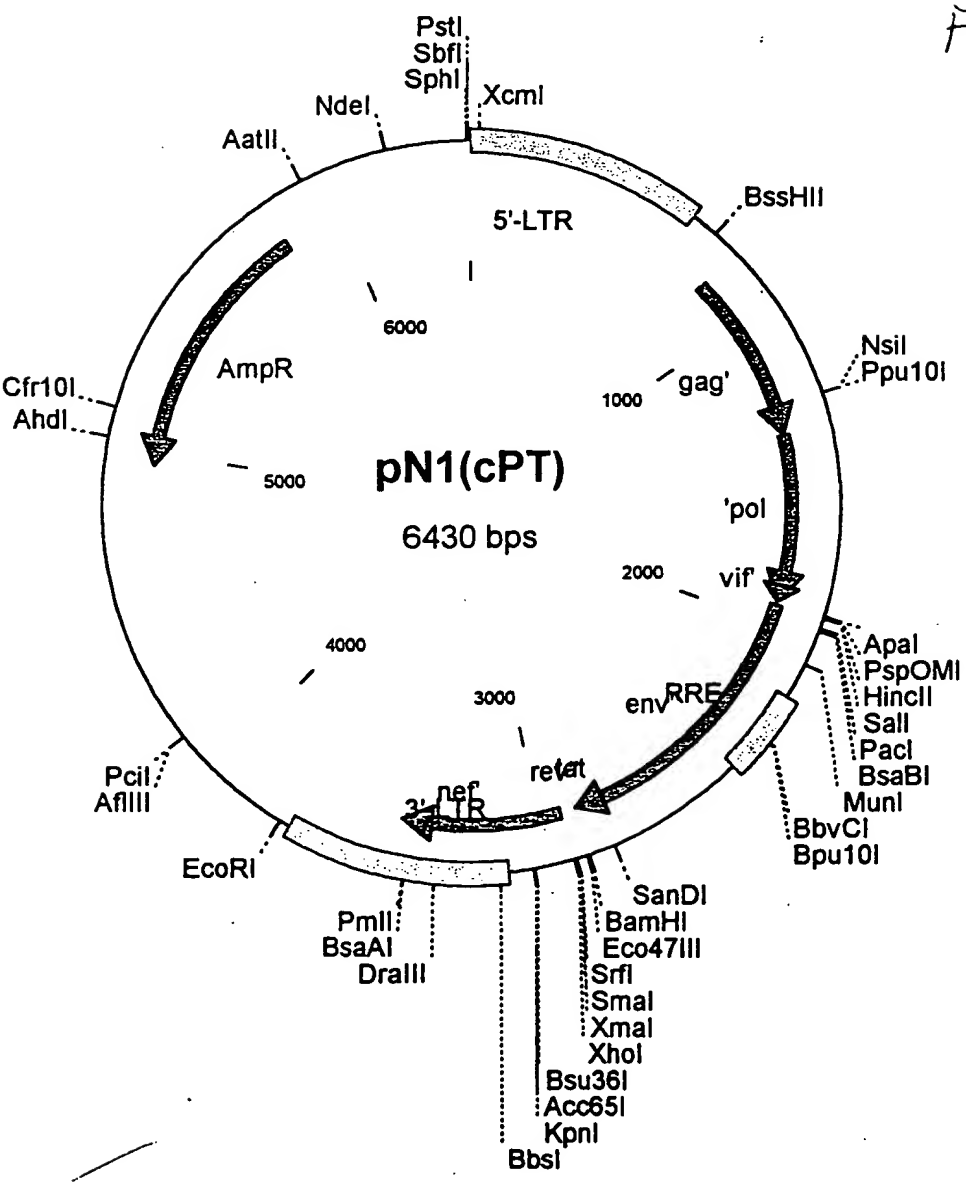

PstI
SbfI
SphI
XcmI
NdeI
AatII
BssHII
5'-LTR
6000
AmpR
Cfr10I
AhdI
1000
gag'
NsiI
Ppu10I
5000
pN1(cPT)
6430 bps
'pol
2000
vif'
4000
3000
env
RRE
rev
tat
nef
3'-LTR
ApaI
PspOMI
HincII
SalI
PacI
BsaBI
MunI
BbvCI
Bpu10I
PciI
AflIII
EcoRI
PmlI
BsaAI
DraIII
SanDI
BamHI
Eco47III
SrfI
SmaI
XmaI
XhoI
Bsu36I
Acc65I
KpnI
BbsI

Fig 1B

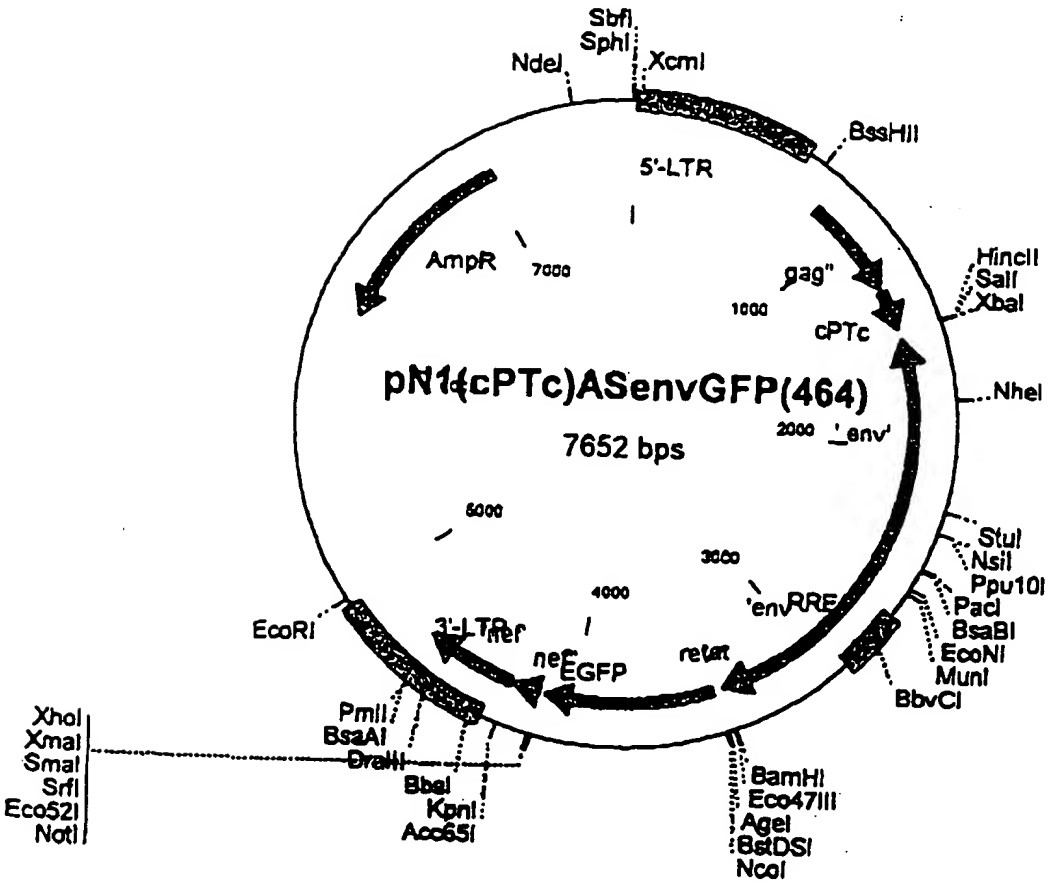
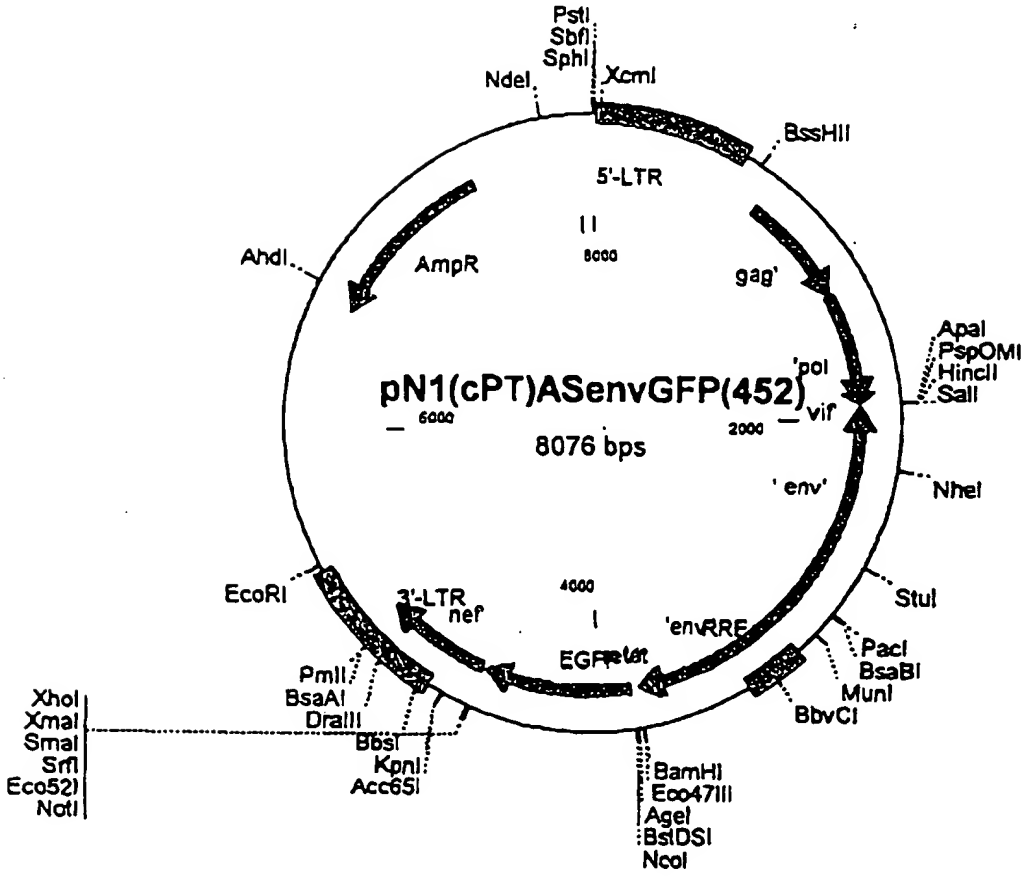

pN14(cPTc)ASenvGFP(464)
7652 bps
5'-LTR
AmpR
7000
gag"
1000
cPTc
2000
'env'
3000
'envRRE
4000
rev
3'-LTR
nef
EGFP
retar
5000
SbfI
SphI
NdeI
XcmI
BssHII
HincII
SalI
XbaI
NheI
StuI
NsiI
Ppu10I
PacI
BsaBI
EcoNI
MunI
BbvCI
BamHI
Eco47III
AgeI
BstDSI
NcoI
EcoRI
PmlI
BsaAI
DraIII
BbeI
KpnI
Acc65I
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI

Fig 1C


pN1(cPT)ASenvGFP(452)
8076 bps
5'-LTR
AmpR
gag'
'pol
vif
' env'
'envRRE
nef
3'-LTR
EGFP
PstI
SbfI
SphI
XcmI
NdeI
BssHII
AhdI
ApaI
PspOMI
HincII
SalI
NheI
StuI
PacI
BsaBI
MunI
BbvCI
EcoRI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BamHI
Eco47III
AgeI
BstDSI
NcoI

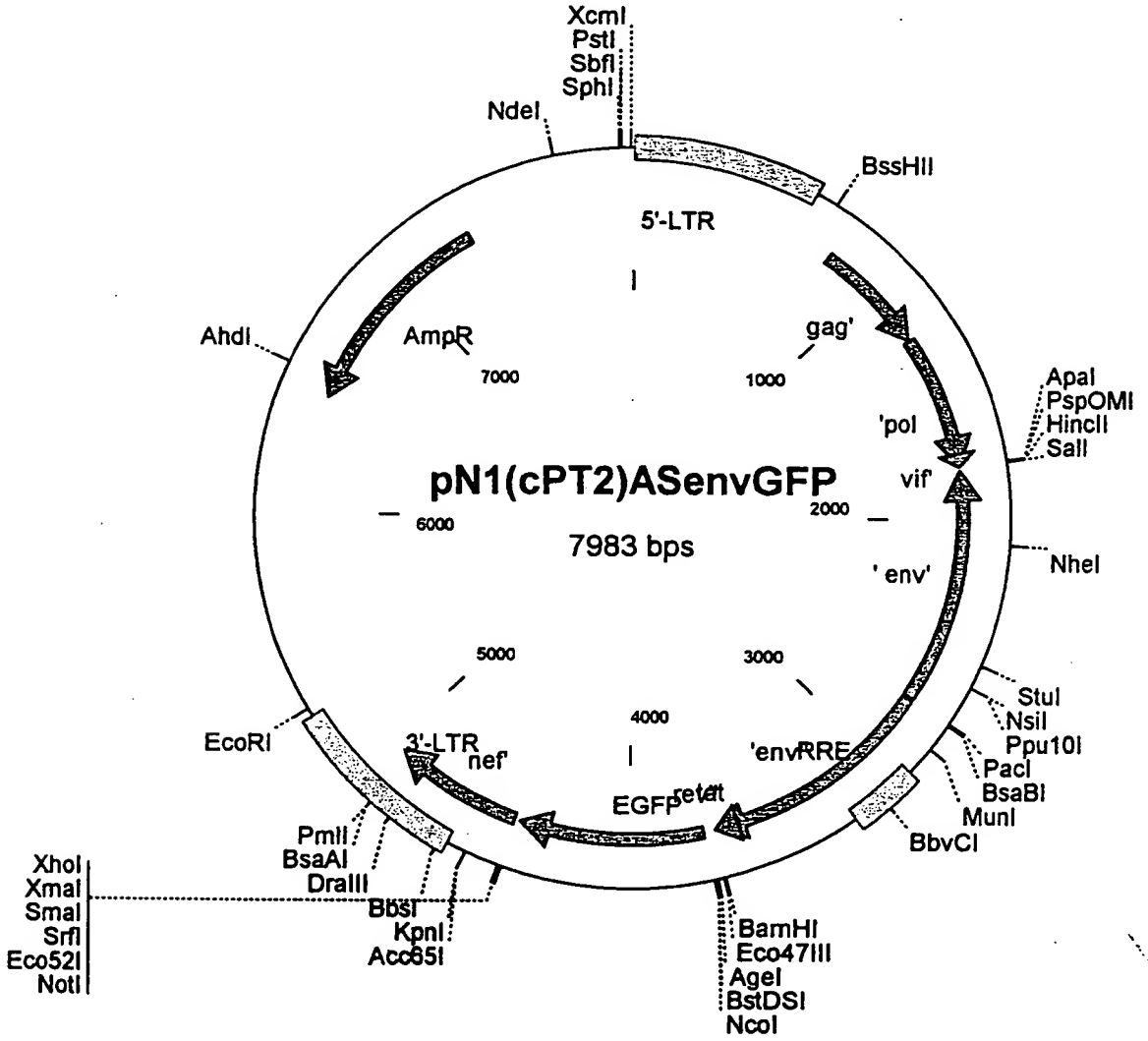

pN1(cPT2)ASenvGFP
7983 bps
XcmI
PstI
SbfI
SphI
NdeI
BssHII
5'-LTR
AhdI
AmpR
gag'
'pol
vif'
' env'
'envRRE
rev
EGFP
3'-LTR
nef'
EcoRI
ApaI
PspOMI
HincII
SalI
NheI
StuI
NsiI
Ppu10I
PacI
BsaBI
MunI
BbvCI
BamHI
Eco47III
AgeI
BstDSI
NcoI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
1000
2000
3000
4000
5000
6000
7000

Fig 1E

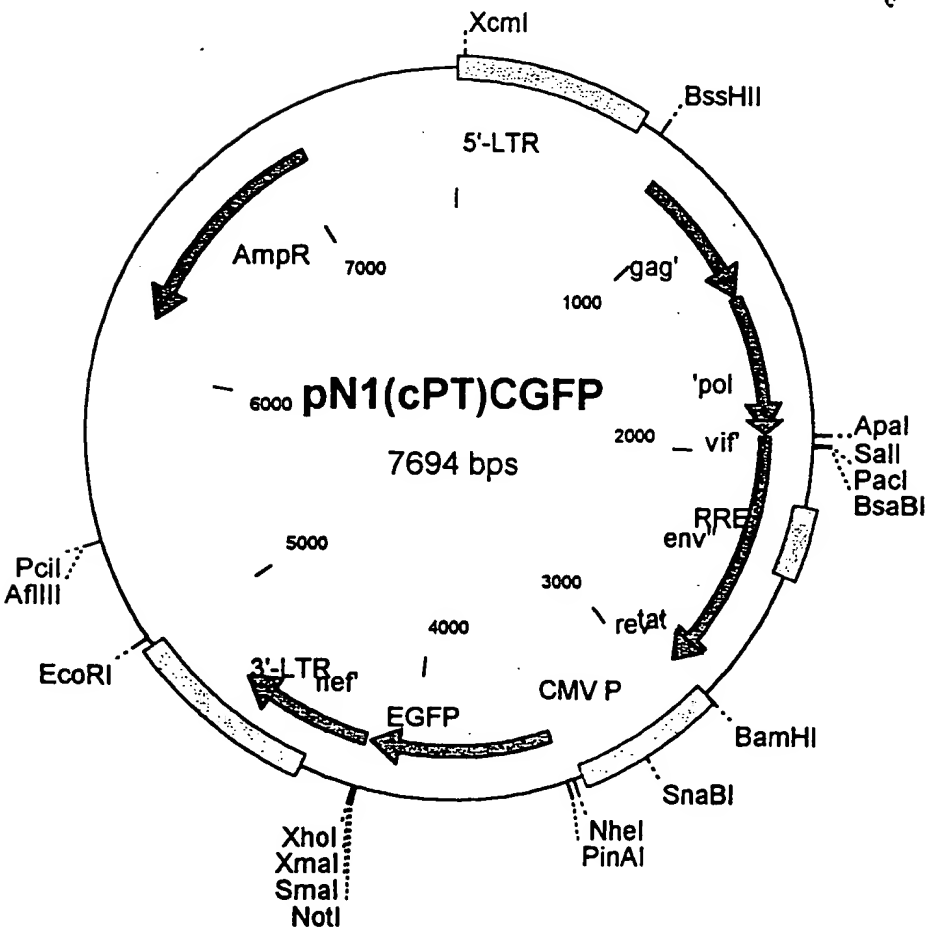

XcmI
BssHII
5'-LTR
AmpR
7000
gag'
1000
6000
pN1(cPT)CGFP
'pol
2000
vif'
7694 bps
ApaI
SalI
PacI
BsaBI
RRE
env'
5000
3000
PciI
AflIII
rev'
4000
3'-LTR
EcoRI
CMV P
EGFP
BamHI
SnaBI
XhoI
XmaI
SmaI
NotI
NheI
PinAI

Fig 1F

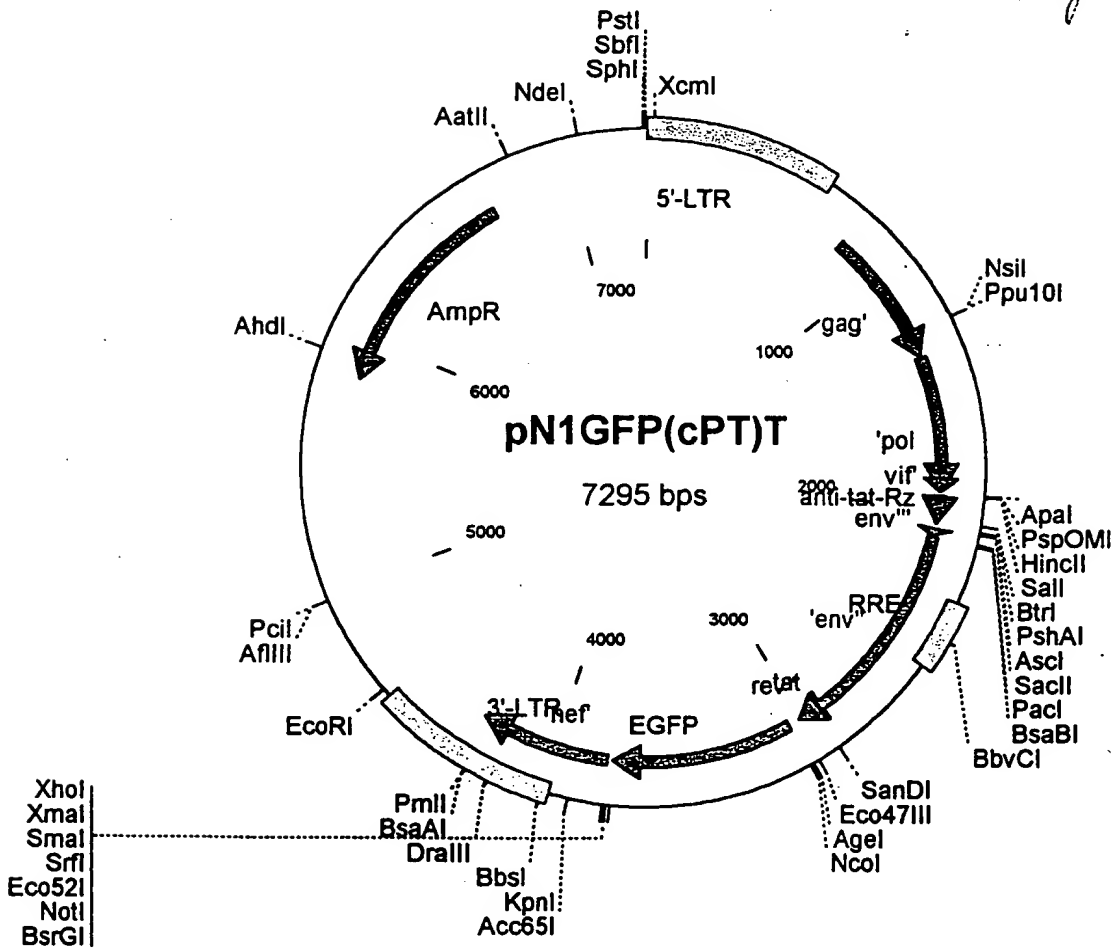
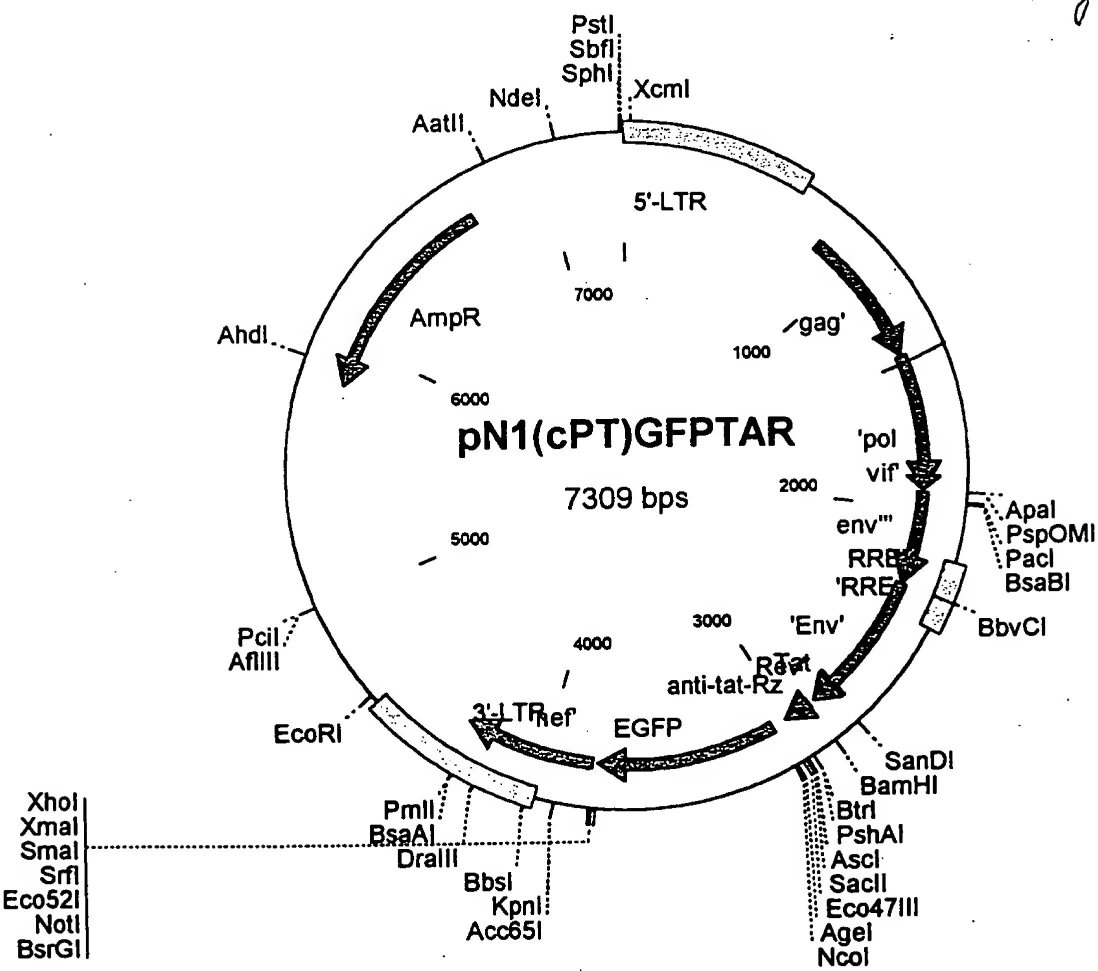

PstI
SbfI
SphI
XcmI
NdeI
AatII
5'-LTR
7000
1000
NsiI
Ppu10I
AmpR
gag'
AhdI
6000
pN1GFP(cPT)T
'pol
vif'
7295 bps
2000
anti-tat-Rz
env'''
ApaI
PspOMI
HincII
SalI
BtrI
PshAI
AscI
SacII
PacI
BsaBI
BbvCI
5000
RRE
'env'
3000
PciI
AflIII
4000
rev
tat
3'-LTR
nef
EGFP
EcoRI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
SanDI
Eco47III
AgeI
NcoI

Fig 1G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7309 bps
5'-LTR
gag'
'pol
vif'
env'''
RRE
'RRE
'Env'
Rev
Tat
anti-tat-Rz
EGFP
nef'
3'-LTR
AmpR
1000
2000
3000
4000
5000
6000
7000
PstI
SbfI
SphI
XcmI
NdeI
AatII
AhdI
PciI
AflIII
EcoRI
ApaI
PspOMI
PacI
BsaBI
BbvCI
SanDI
BamHI
BtrI
PshAI
AscI
SacII
Eco47III
AgeI
NcoI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI

Fig 1H

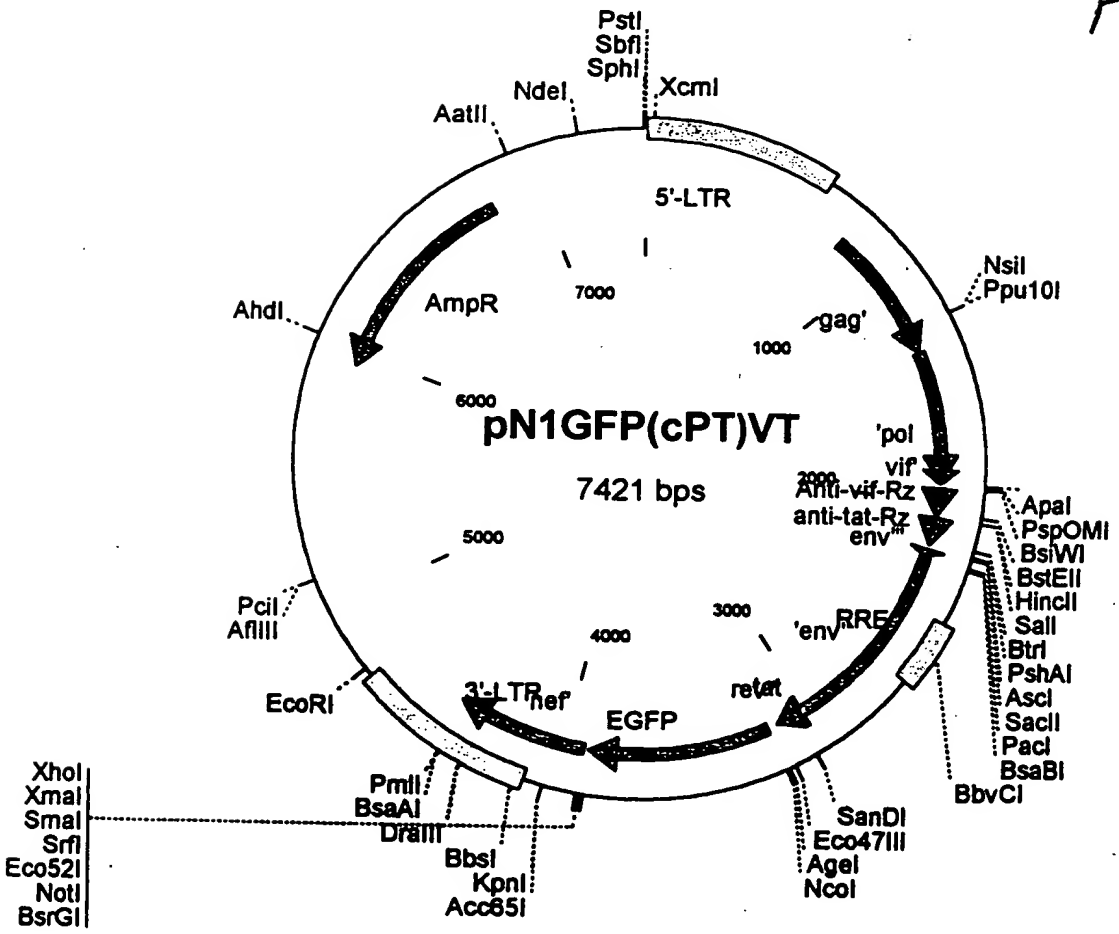
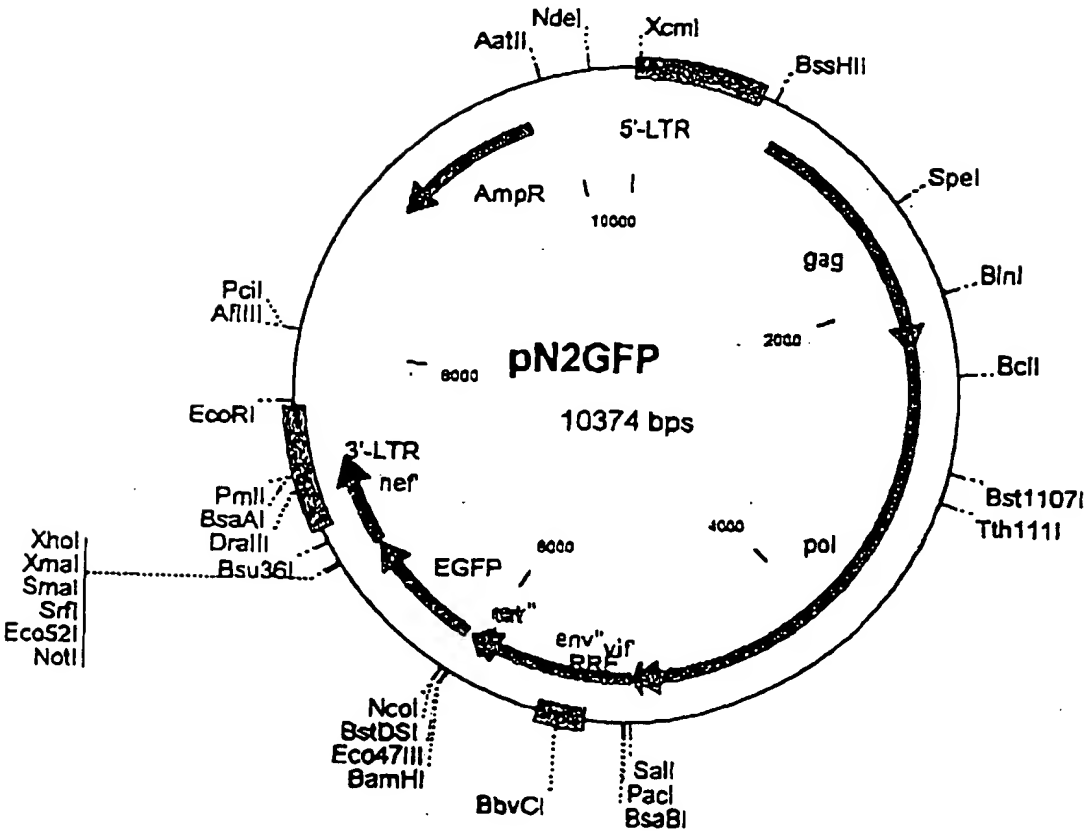

pN1GFP(cPT)VT
7421 bps
5'-LTR
'gag'
'pol
vif'
Anti-vif-Rz
anti-tat-Rz
env'''
'env'
RRE
rev
EGFP
3'-LTR
ref'
AmpR
7000
1000
2000
3000
4000
5000
6000
PstI
SbfI
SphI
XcmI
NdeI
AatII
NsiI
Ppu10I
ApaI
PspOMI
BsiWI
BstEII
HincII
SalI
BtrI
PshAI
AscI
SacII
PacI
BsaBI
BbvCI
SanDI
Eco47III
AgeI
NcoI
KpnI
Acc65I
BbsI
PmlI
BsaAI
DraIII
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI
EcoRI
PciI
AflIII
AhdI

Fig 1I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10374 bps
5'-LTR
AmpR
gag
pol
env
vif
RRE
rev
EGFP
3'-LTR
nef
NdeI
AatII
XcmI
BssHII
SpeI
BlnI
BclI
Bst1107I
Tth111I
PciI
AflIII
EcoRI
PmlI
BsaAI
DraIII
Bsu36I
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
NcoI
BstDSI
Eco47III
BamHI
BbvCI
SalI
PacI
BsaBI

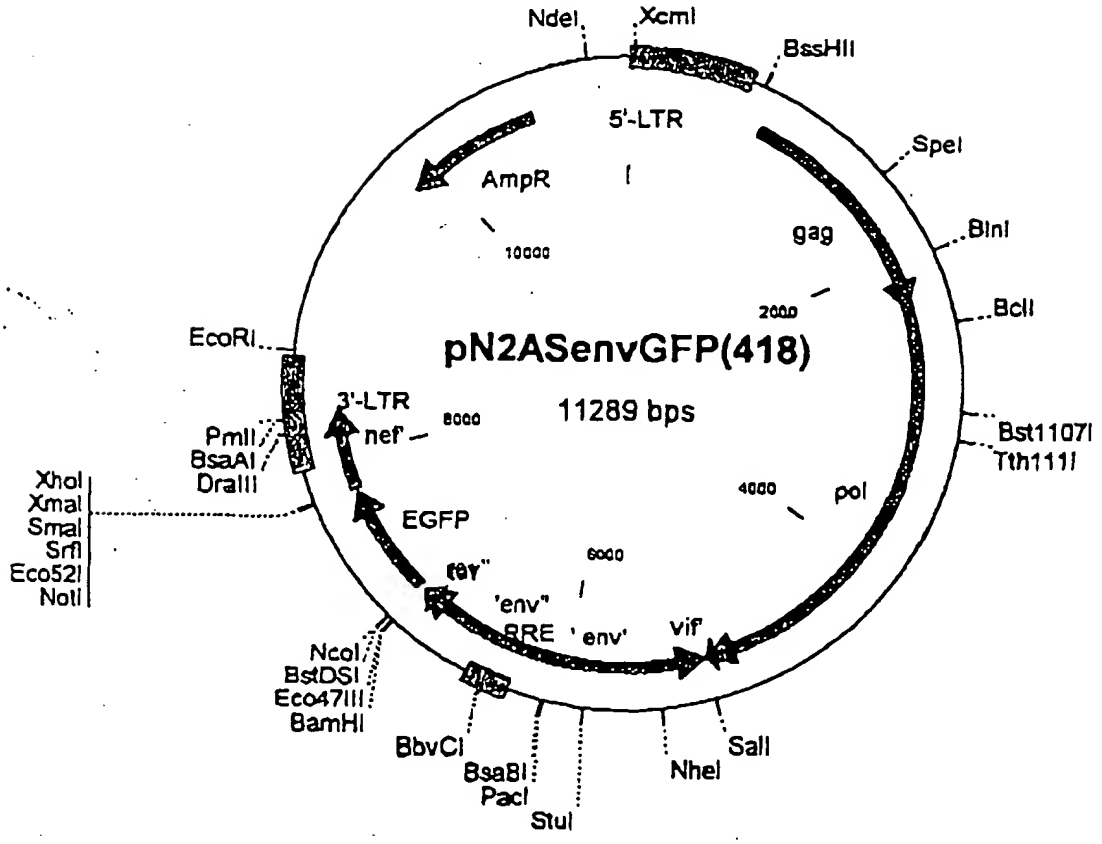
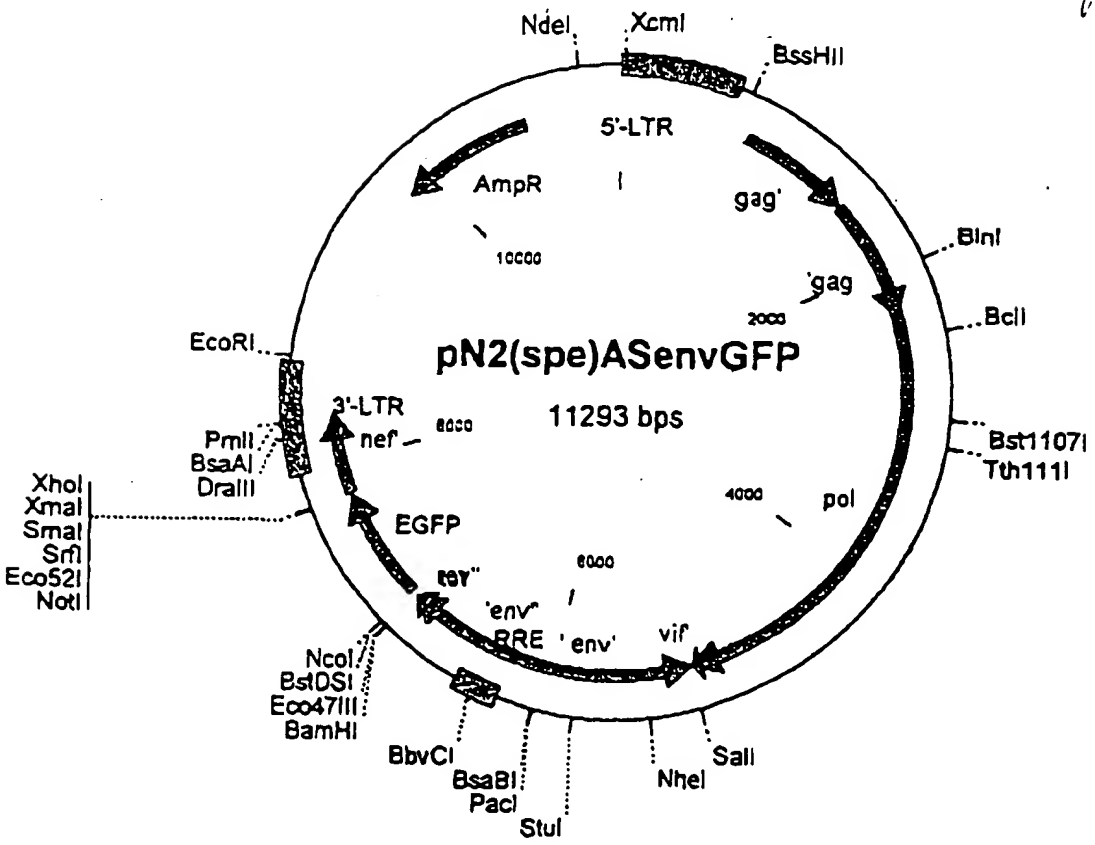


Fig 1J

Fig 1K

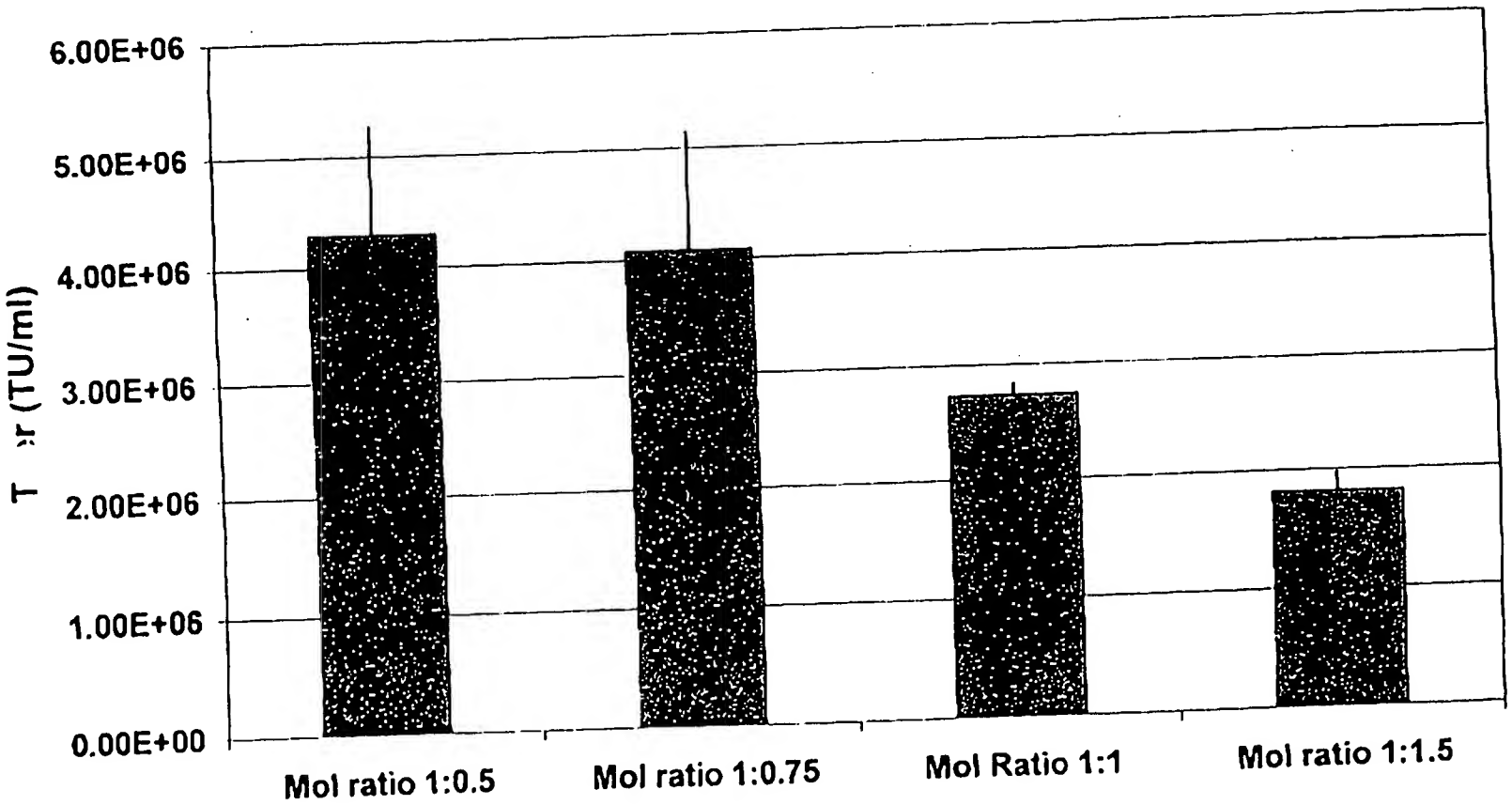

NdeI
XcmI
BssHII
5'-LTR
AmpR
gag'
BlnI
'gag
BclI
EcoRI
pN2(spe)ASenvGFP
11293 bps
3'-LTR
nef
PmlI
BsaAI
DraIII
Bst1107I
Tth111I
pol
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
EGFP
'env'
RRE
'env'
vif
NcoI
BstDSI
Eco47III
BamHI
BbvCI
BsaBI
PacI
StuI
NheI
SalI

A	+105	GTGTGCCCGTCTG	+117
B		. . . . . AC . . .	
A	+118	TTGTGTGACTCTG	+130
B		. . . . .	
A	+131	GTAACTAGAGATC	+143
B		. C . G . . . . . A .	

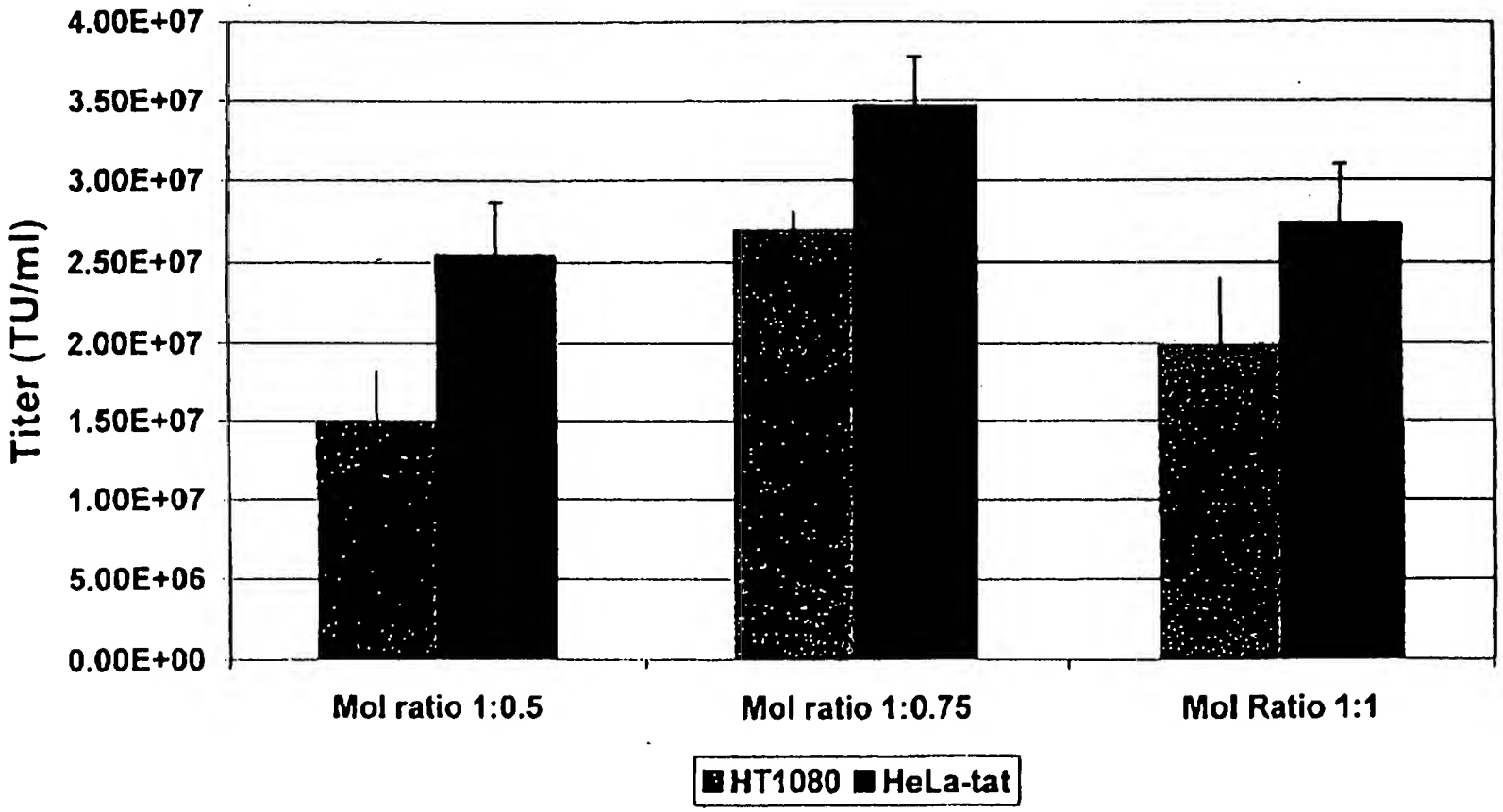
FIG. 2

Title: IMPROVED CONDITIONALLY REPLICATING VECTORS FOR INHIBITING VIRAL INFECTIONS
First Inventor: Laurent HUMEAU et al
Application No.: 09/819,401 - Docket No. 397272000700
Sheet 13 of 49

Ratio Optimization for pN1(cPTC)ASenvGFP Vector



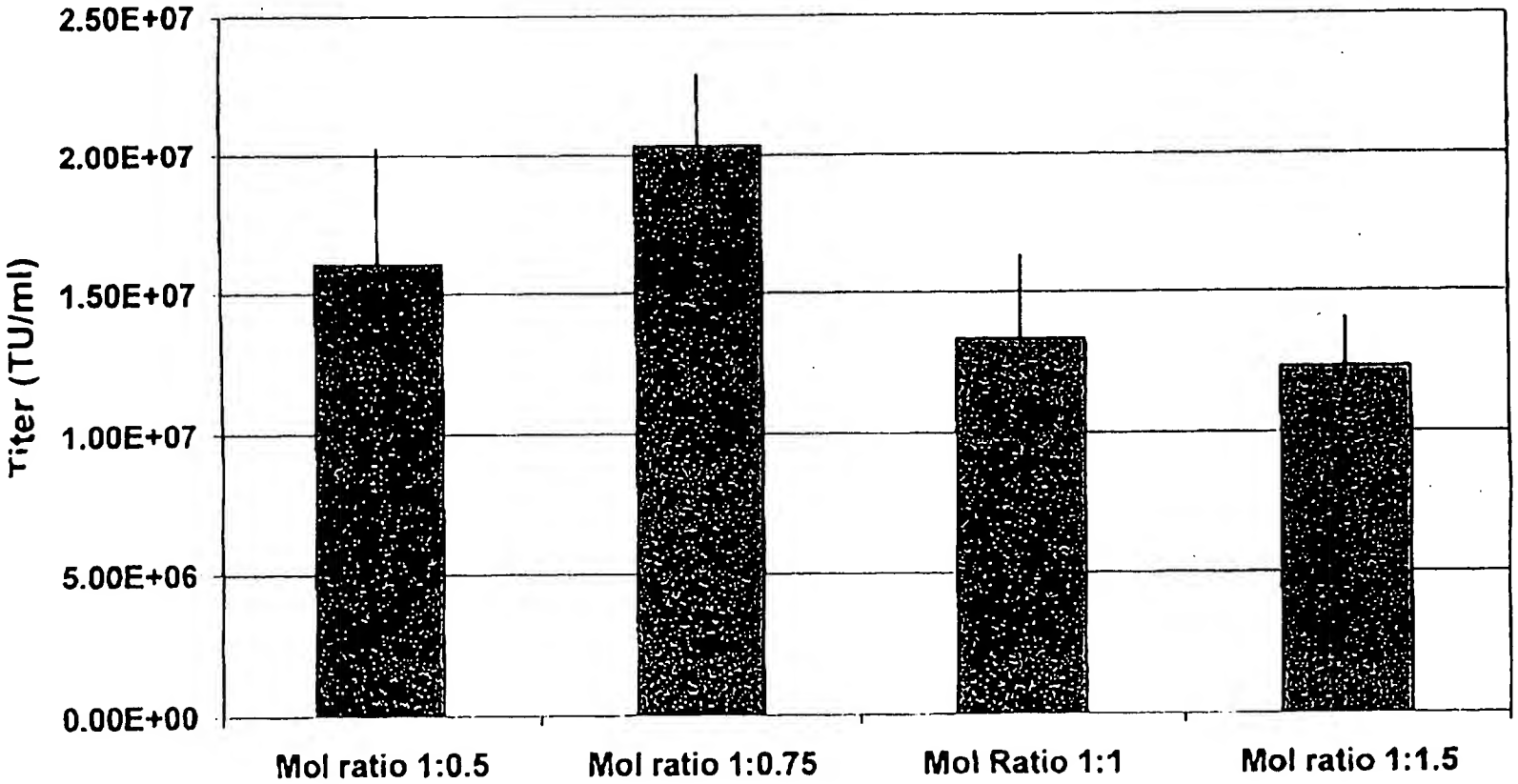
Ratio Optimization for pN1(cPT)GFP Vectors



Title: IMPROVED CONDITIONALLY REPLICATING VECTORS FOR INHIBITING VIRAL INFECTIONS
First Inventor: Laurent HUMEAU et al
Application No.: 09/819,401 - Docket No. 39727200700
Sheet 14 of 49

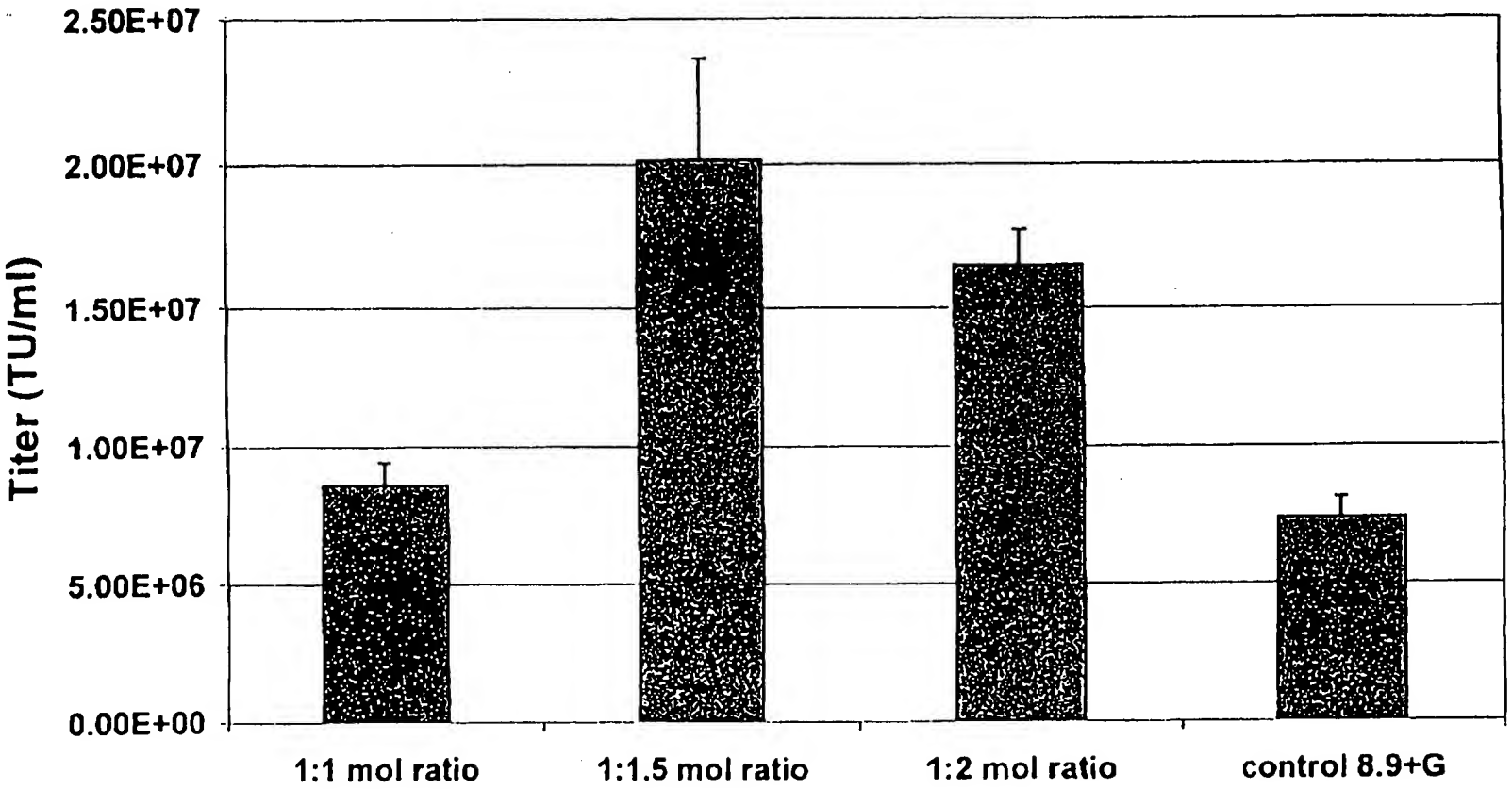
3C

Ratio Optimization for pN1(cPT2)ASenvGFP Vector



3D

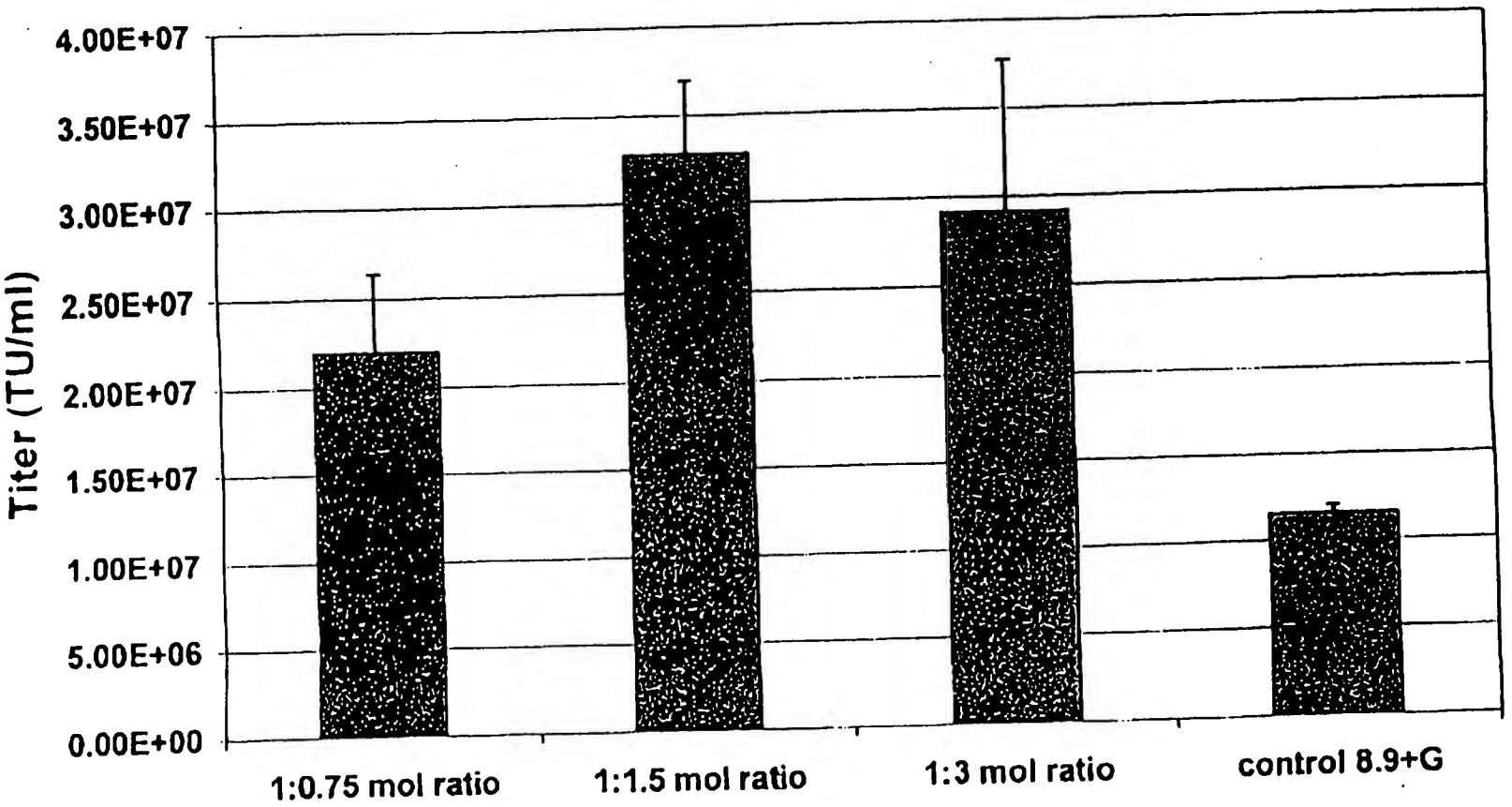
Best Vector to Packaging Ratio for pN1cGFP Vector



Title: IMPROVED CONDITIONALLY REPLICATING VECTORS FOR INHIBITING VIRAL INFECTIONS
First Inventor: Laurent HUMEAU et al
Application No.: 09/819,401 - Docket No. 397272000700
Sheet 16 of 49

3E

Optimiztion of vector to packaging ratio for pN2cGFP



Title: IMPROVED CONDITIONALLY REPLICATING VECTORS FOR INHIBITING VIRAL INFECTIONS
First Inventor: Laurent HUMEAU et al
Application No.: 09/819,401 - Docket No. 397272000700
Sheet 17 of 49

Fig 4A

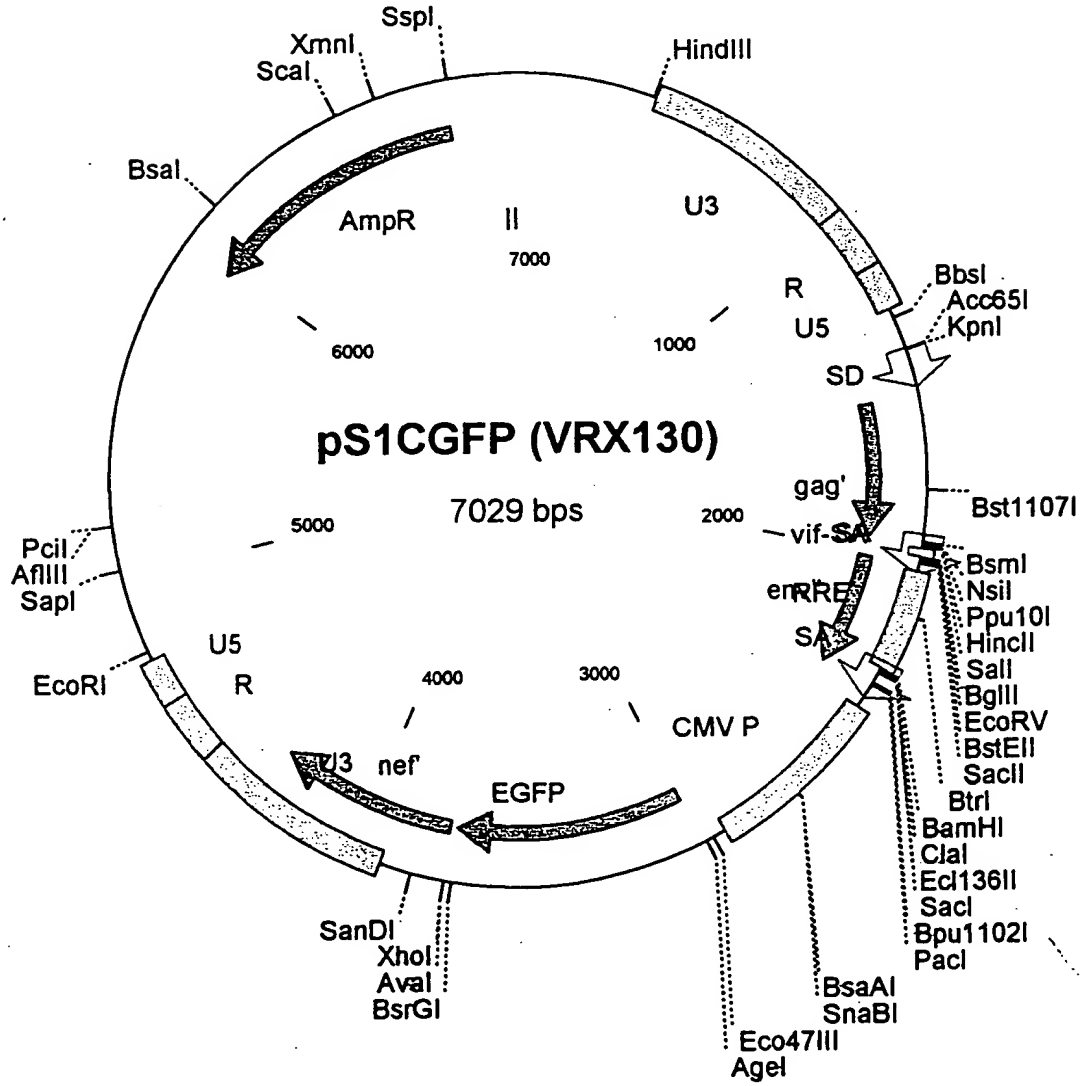
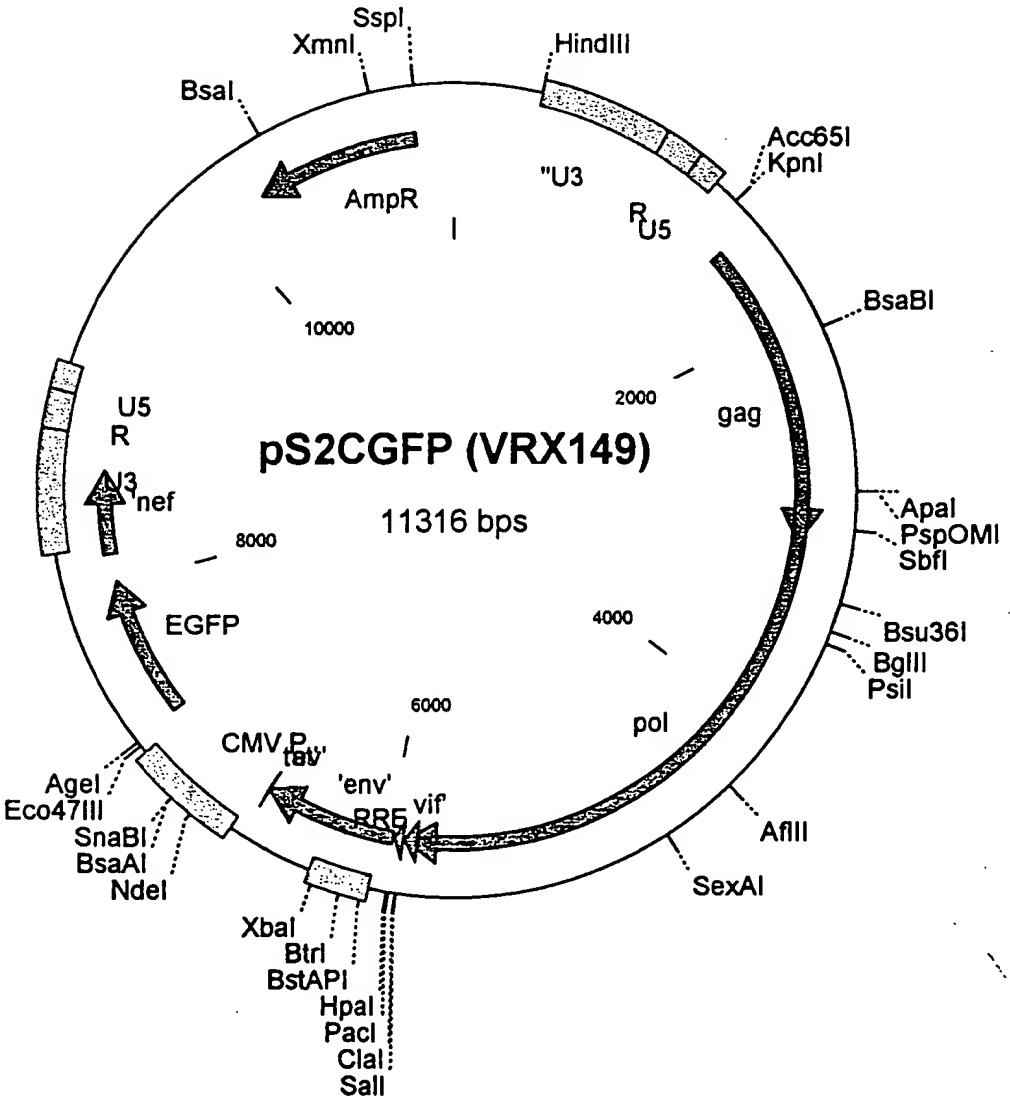
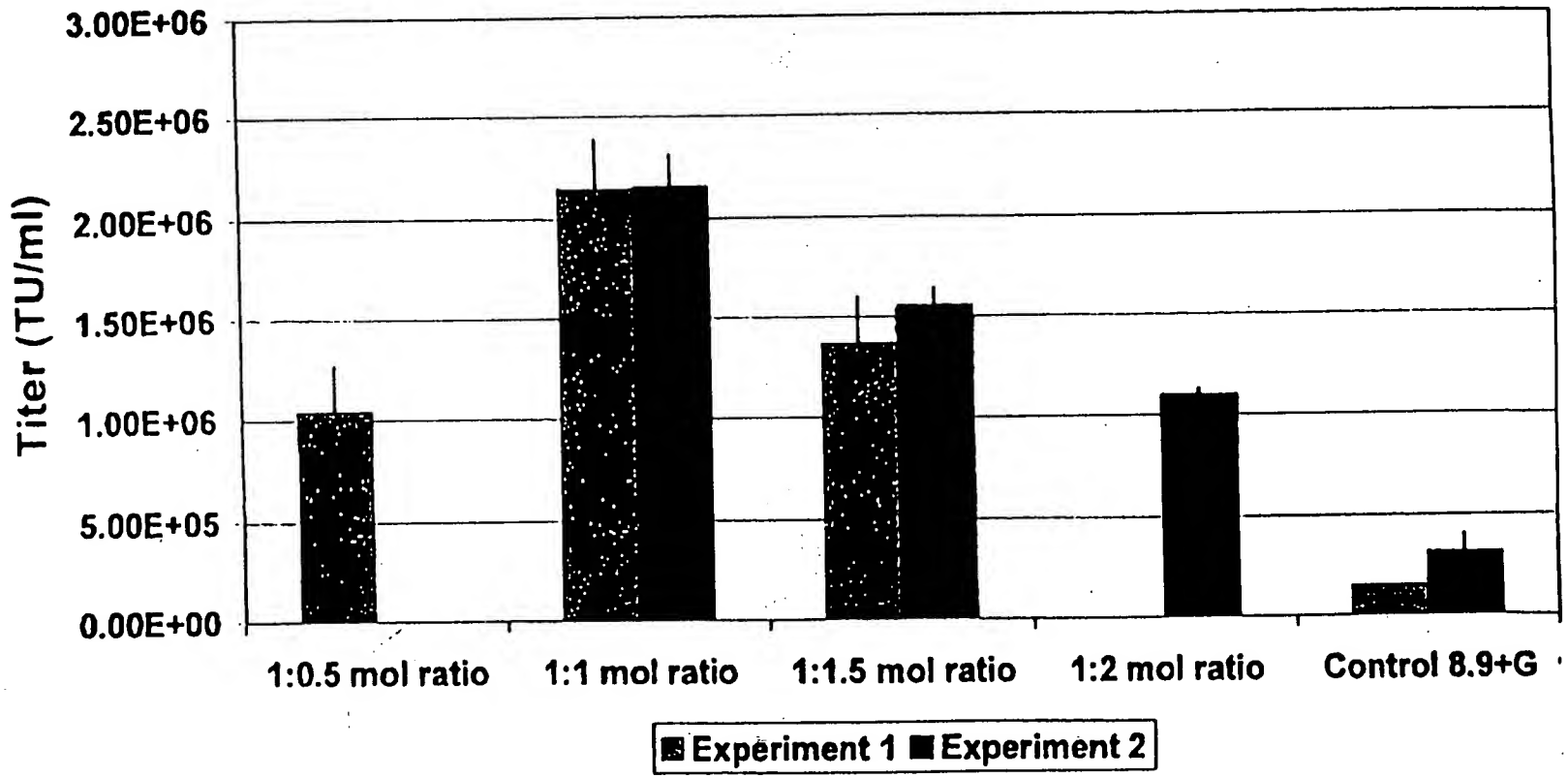

pS1CGFP (VRX130)
7029 bps
SspI
XmnI
ScaI
HindIII
BsaI
AmpR
ll
U3
7000
R
U5
1000
6000
BbsI
Acc65I
KpnI
SD
gag'
2000
Bst1107I
5000
vif-SA
PciI
AflIII
SapI
enRRE
SA
BsmI
NsiI
Ppu10I
HincII
SalI
BglII
EcoRV
BstEII
SacII
BtrI
BamHI
ClaI
Ecl136II
SacI
Bpu1102I
PacI
U5
R
EcoRI
4000
3000
CMV P
U3
nef'
EGFP
SanDI
XhoI
AvaI
BsrGI
BsaAI
SnaBI
Eco47III
AgeI

Fig 4B


pS2CGFP (VRX149)
11316 bps
SspI
XmnI
HindIII
BsaI
Acc65I
KpnI
"U3
R
U5
AmpR
BsaBI
10000
2000
gag
U5
R
U3
nef
ApaI
PspOMI
SbfI
8000
EGFP
4000
Bsu36I
BglII
PsiI
6000
pol
CMV P
tat
'env'
vif'
RRE
AgeI
Eco47III
SnaBI
BsaAI
NdeI
AflII
SexAI
XbaI
BtrI
BstAPI
HpaI
PacI
ClaI
SalI

Ratio Optimization for Packaging of pS1cGFP vectors.



Title: IMPROVED CONDITIONALLY REPLICATING VECTORS FOR INHIBITING VIRAL INFECTIONS
First Inventor: Laurent HUMEAU et al
Application No.: 09/819,401 - Docket No. 397272000700
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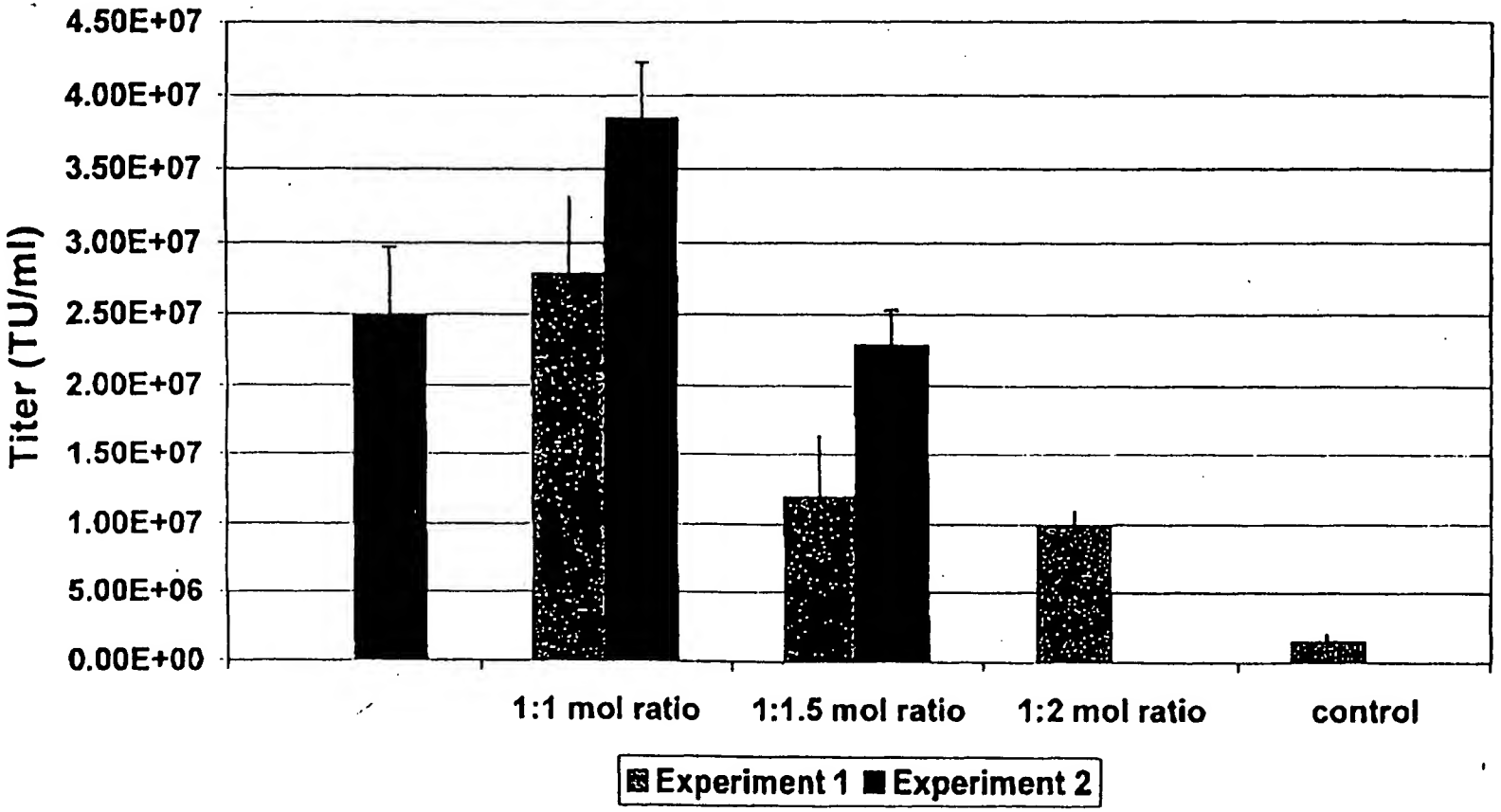
Optimiztion of vector to packaging ratio for pS2cGFP



Title: IMPROVED CONDITIONALLY REPLICATING VECTORS FOR INHIBITING VIRAL INFECTIONS
Inventor: Laurent HUMEAU et al
Application No.: 09/819,401 - Docket No. 39727200700
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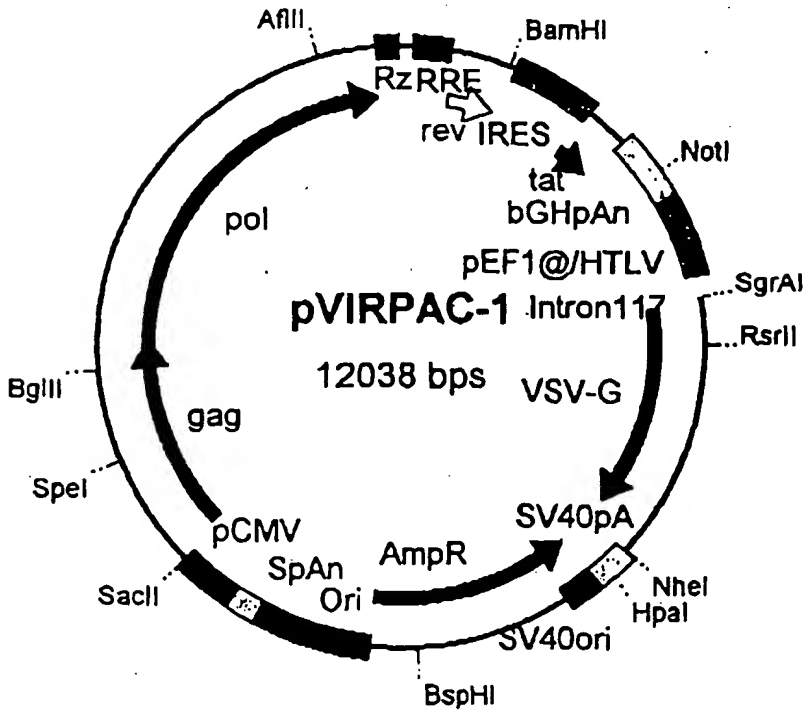
Packaging Construct



New features:

- First 42 nt of gag are degenerated.
- Tat and rev represented as cDNA.
- First 208 nt of rev and last 183 nt of tat are degenerated.
- RRE from HIV-2 is used instead of HIV-1 RRE.

These features eliminate almost any homology with the vector plasmid, make system safer.

- Anti-U5 ribozyme is expressed within gag/pol/RRE cassette, further improving safety.
- Gag/pol/rev/tat/RRE cassette and VSV-G expressed from the same plasmid. This feature may enhance packaging efficiency and titers of the vectors.

Fig. 6B Packaging Plasmid for Second Generation Vectors

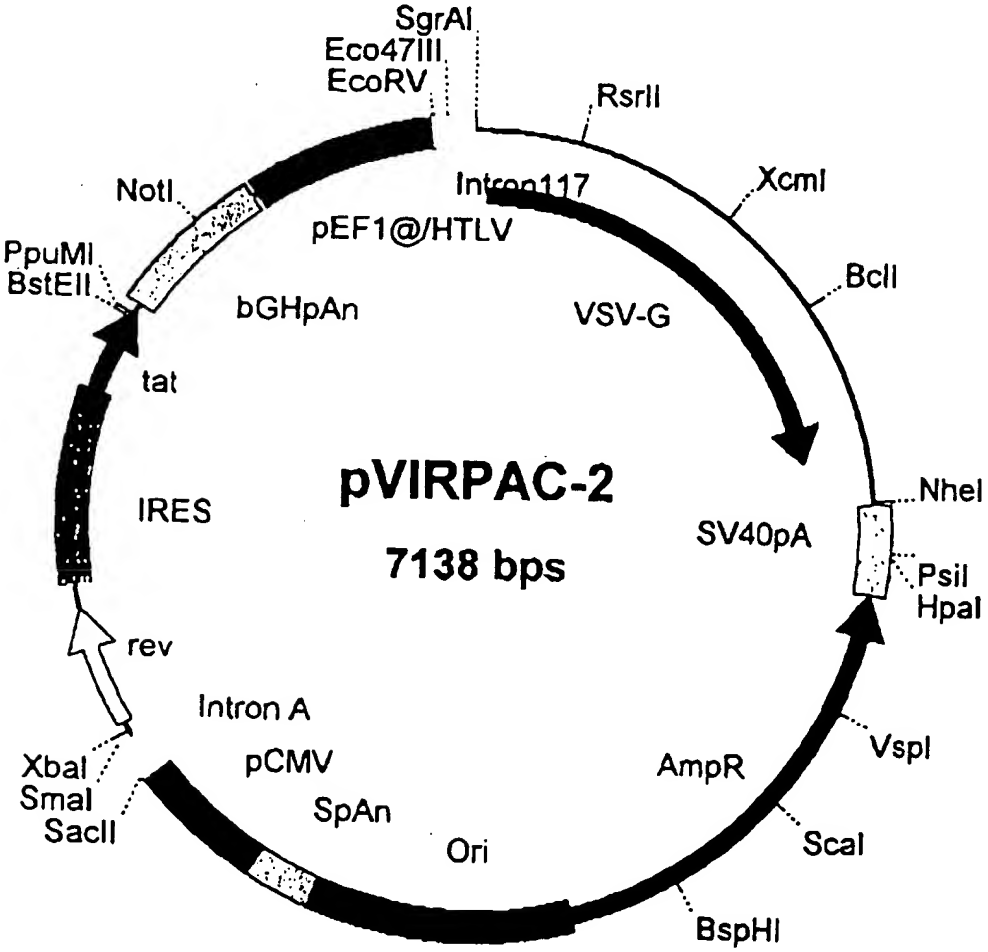


Fig. 6C Packaging Plasmid for First Generation Vectors

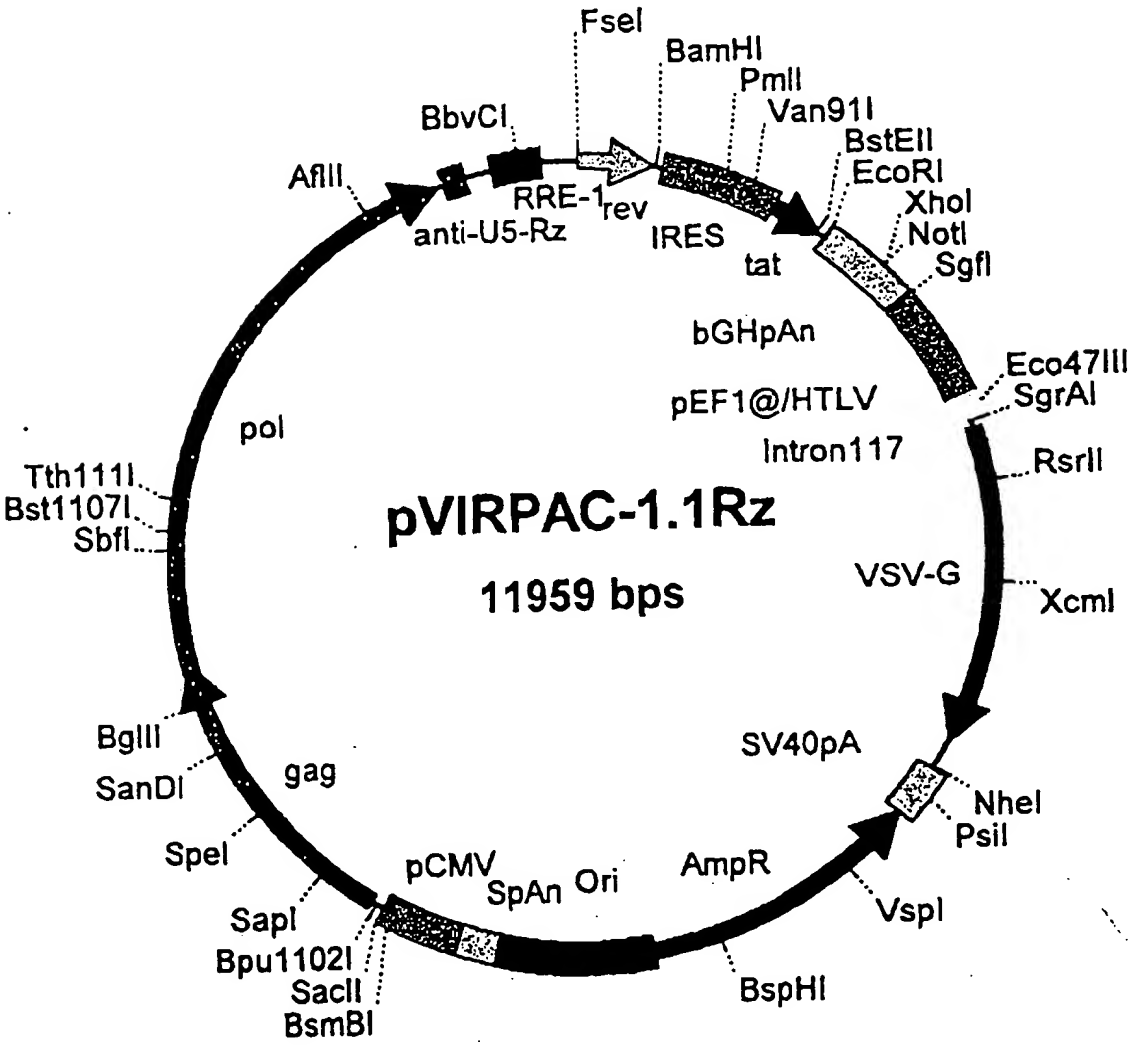


Fig 6D

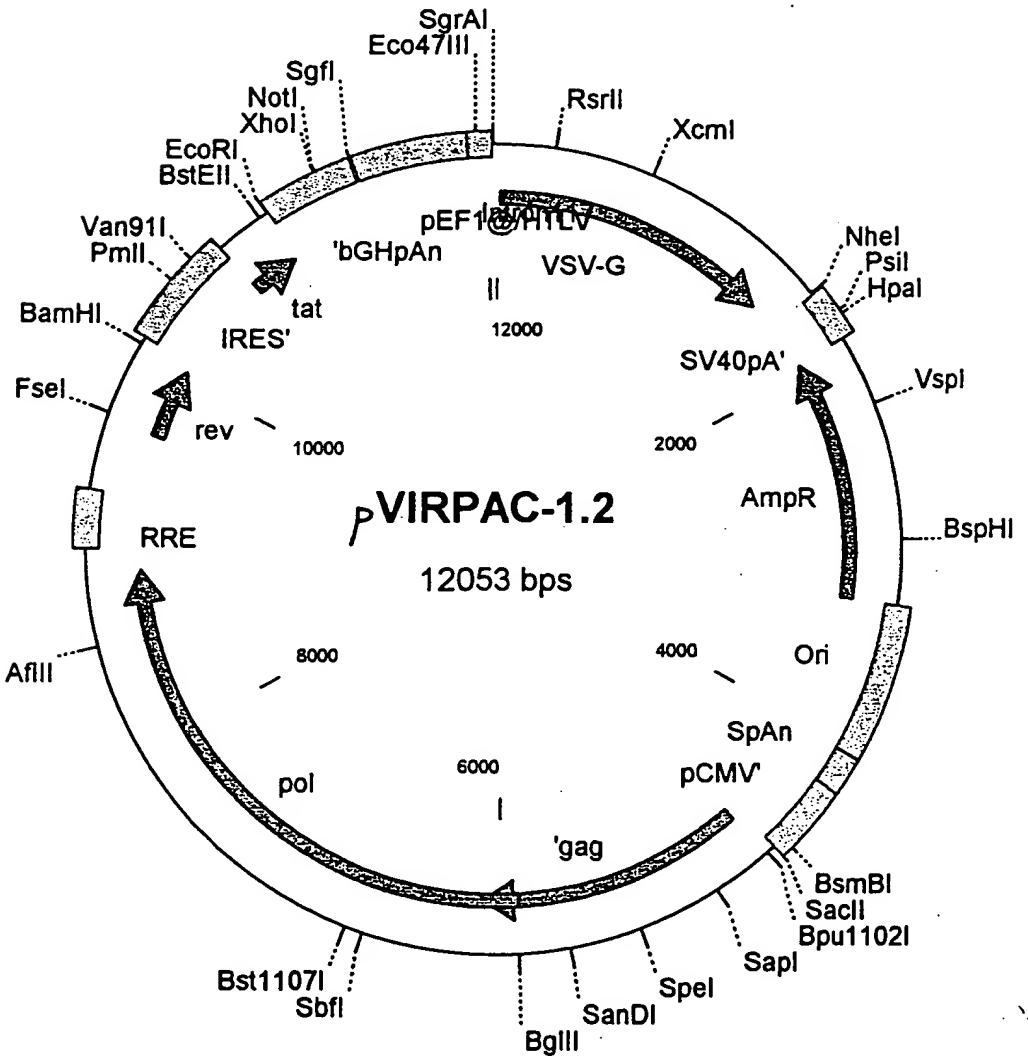

pVIRPAC-1.2
12053 bps
SgrAI
Eco47III
SgfI
NotI
XhoI
EcoRI
BstEII
Van91I
PmlI
BamHI
FseI
AflII
RsrII
XcmI
NheI
PsiI
HpaI
VspI
BspHI
BsmBI
SacII
Bpu1102I
SapI
SpeI
SanDI
BglII
Bst1107I
SbfI
pEF1α/HTLV
'bGHpAn
VSV-G
tat
IRES'
rev
RRE
pol
'gag
pCMV'
SpAn
Ori
AmpR
SV40pA'
2000
4000
6000
8000
10000
12000

Fig 6E

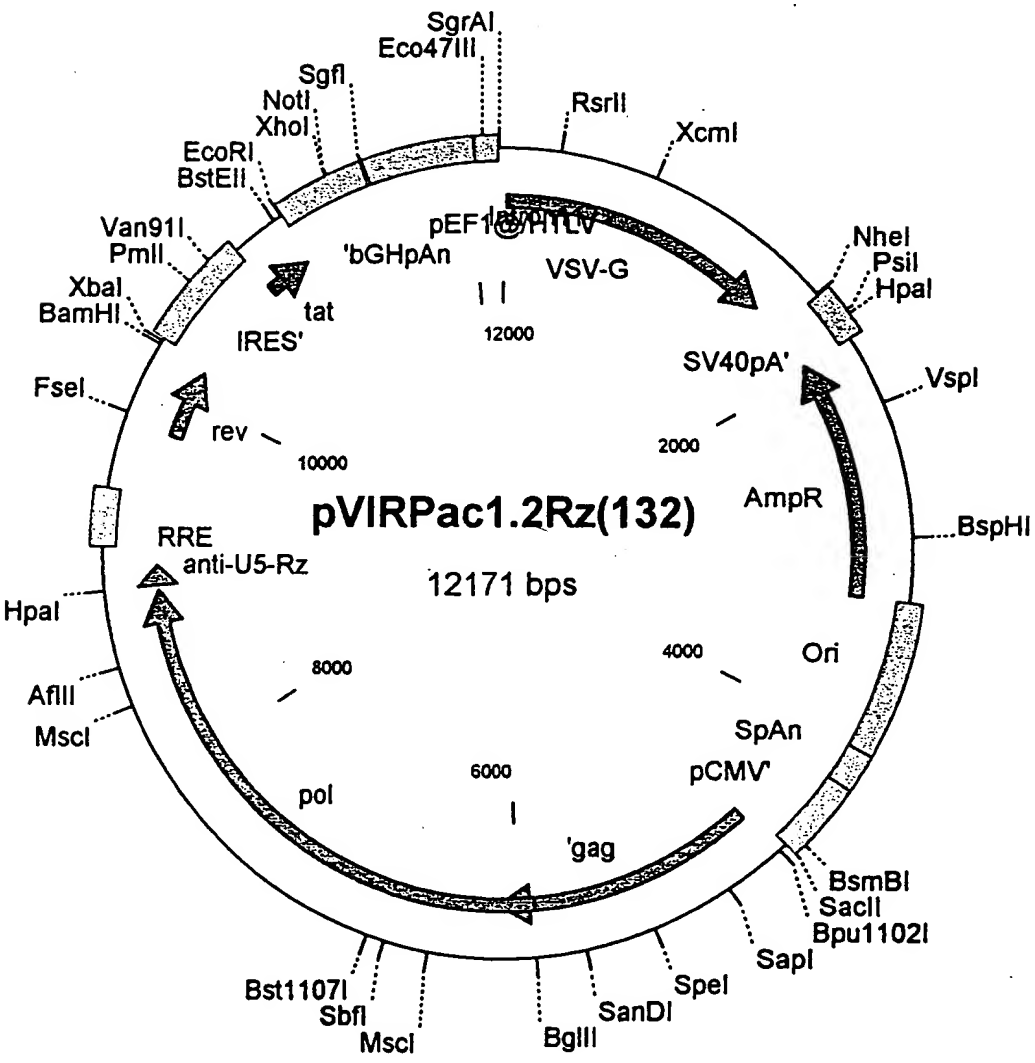
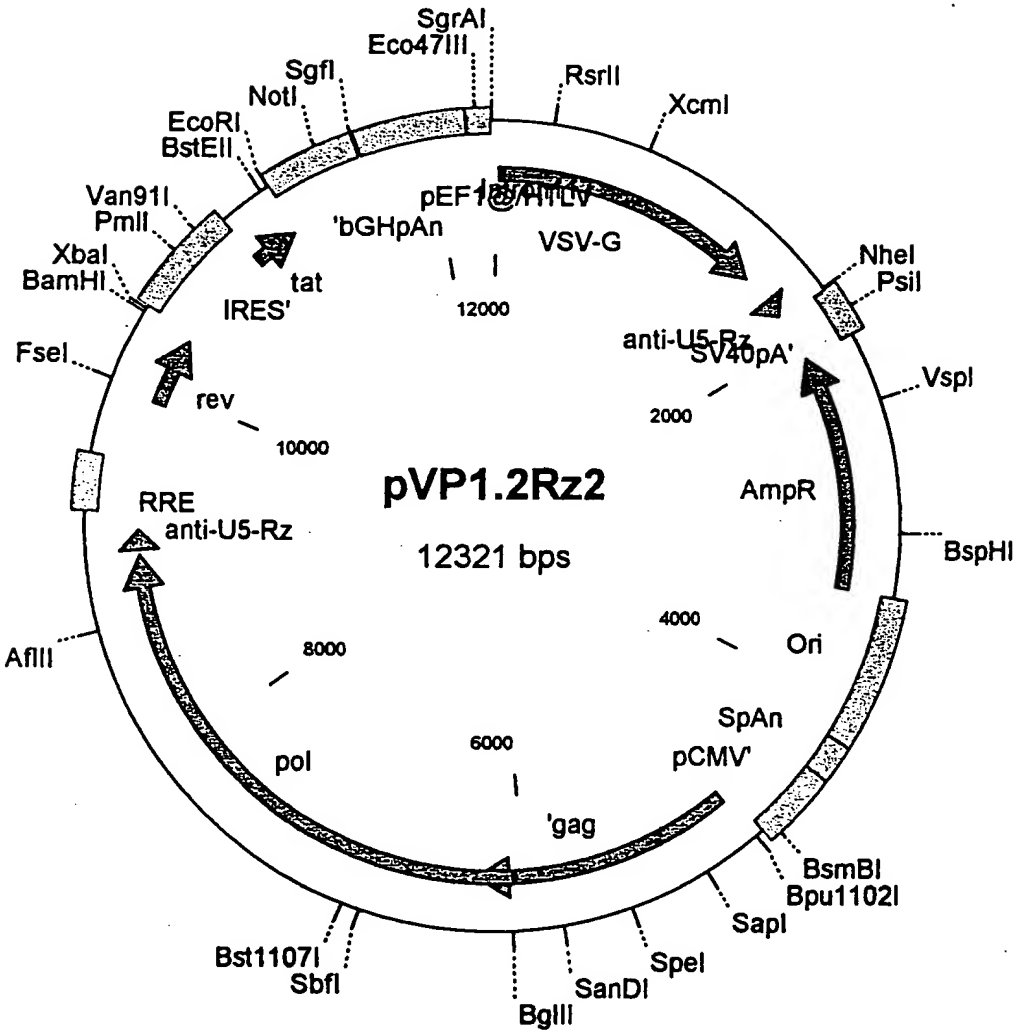


Fig 6F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12321 bps
SgrAI
Eco47III
SgfI
NotI
EcoRI
BstEII
Van91I
PmlI
XbaI
BamHI
FseI
AflII
RsrII
XcmI
NheI
PsiI
VspI
BspHI
BsmBI
Bpu1102I
SapI
SpeI
SanDI
BglII
Bst1107I
SbfI
RRE
anti-U5-Rz
rev
IRES'
tat
'bGHpAn
VSV-G
anti-U5-Rz
SV40pA'
AmpR
Ori
SpAn
pCMV'
'gag
pol
2000
4000
6000
8000
10000
12000

Fig 6G

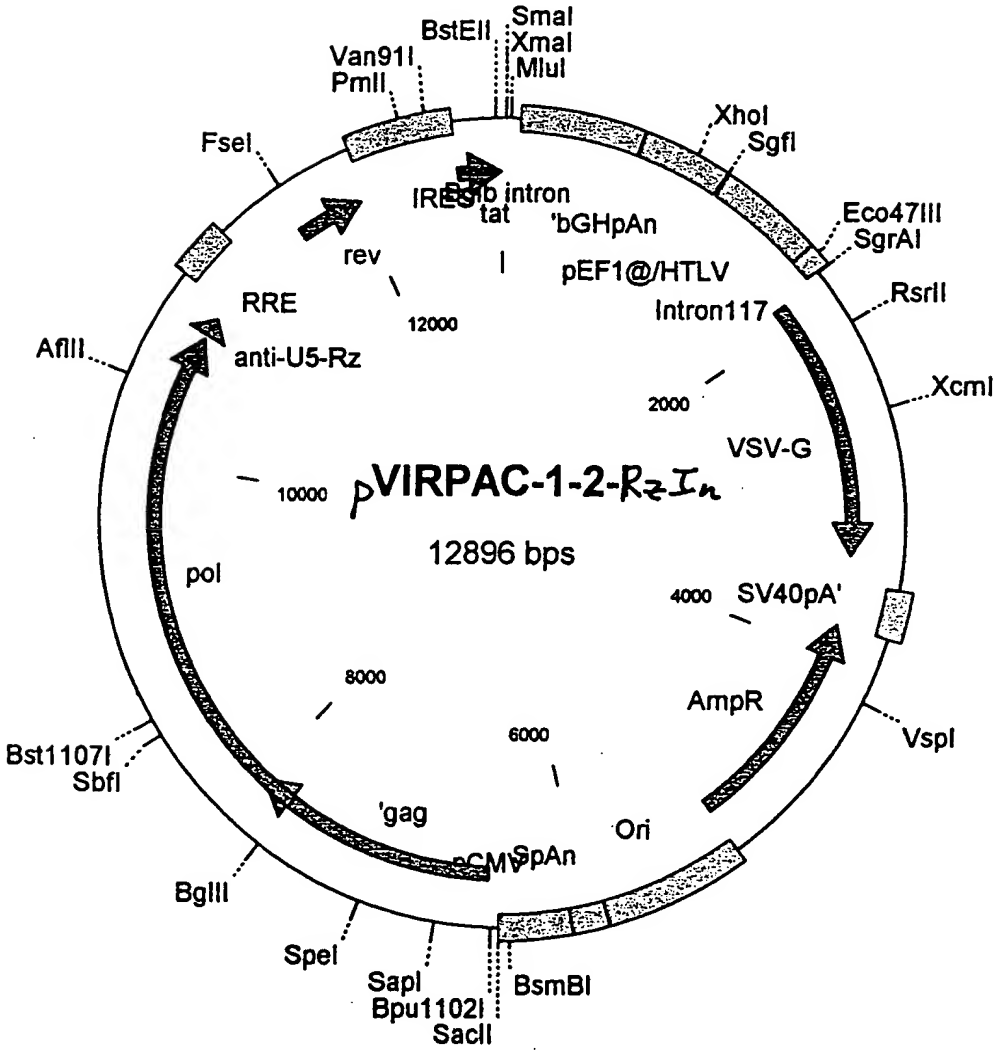
SmaI
XmaI
MluI
BstEII
Van91I
PmlI
FseI
XhoI
SgfI
IRES
Rsb intron
tat
'bGHpAn
Eco47III
SgrAI
rev
pEF1@/HTLV
RRE
12000
Intron117
RsrII
AflII
anti-U5-Rz
2000
XcmI
VSV-G
10000
pVIRPAC-1-2-RzIn
12896 bps
pol
4000
SV40pA'
8000
AmpR
VspI
6000
Bst1107I
SbfI
'gag
Ori
eCMV
SpAn
BglII
SpeI
SapI
Bpu1102I
SacII
BsmBI

Fig 1

Influence of Ribozyme(s) in the Packaging on pN1(cPT)GFP Vector Titers in HeLa-tat Cells

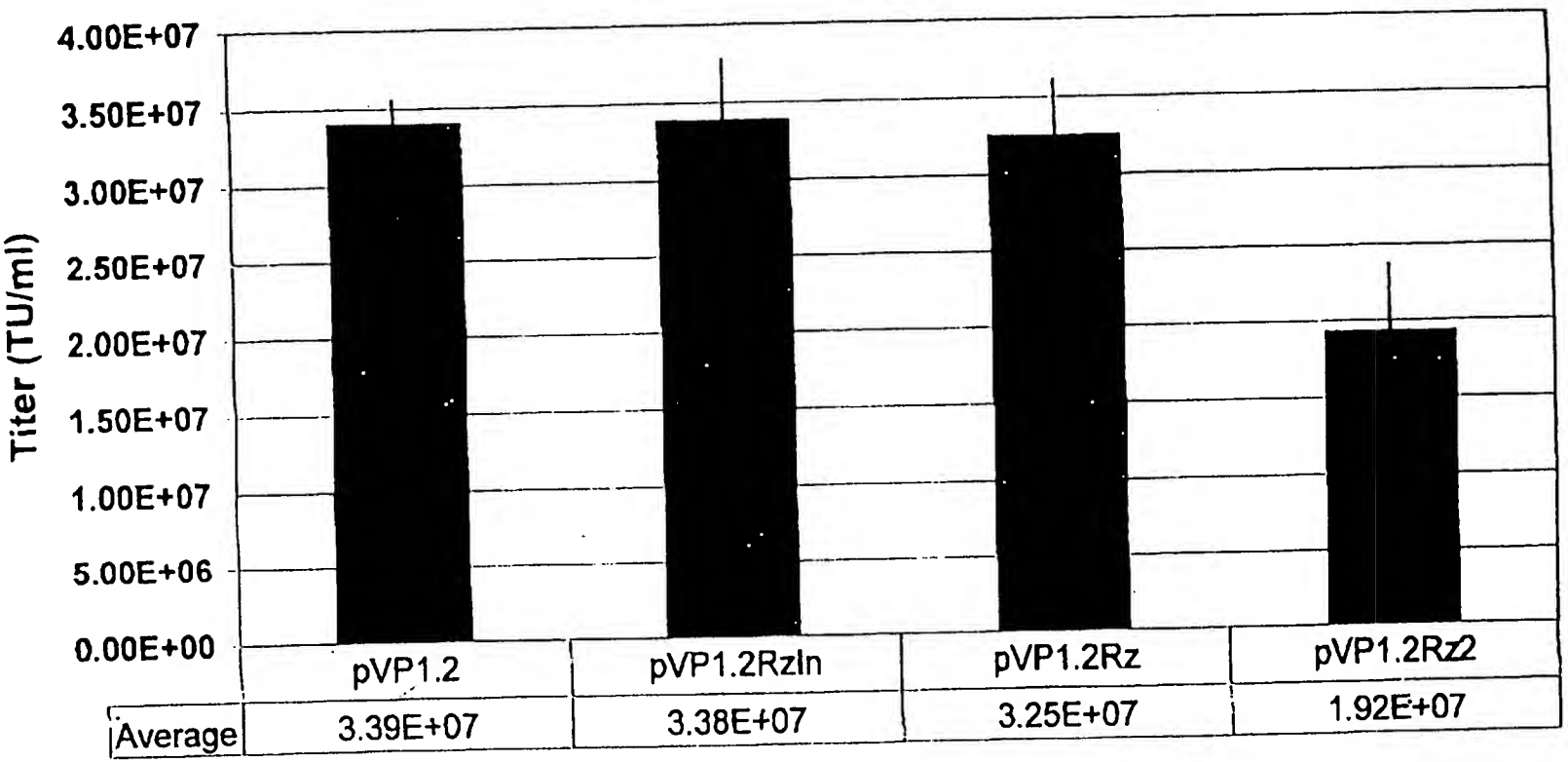

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Fig 8

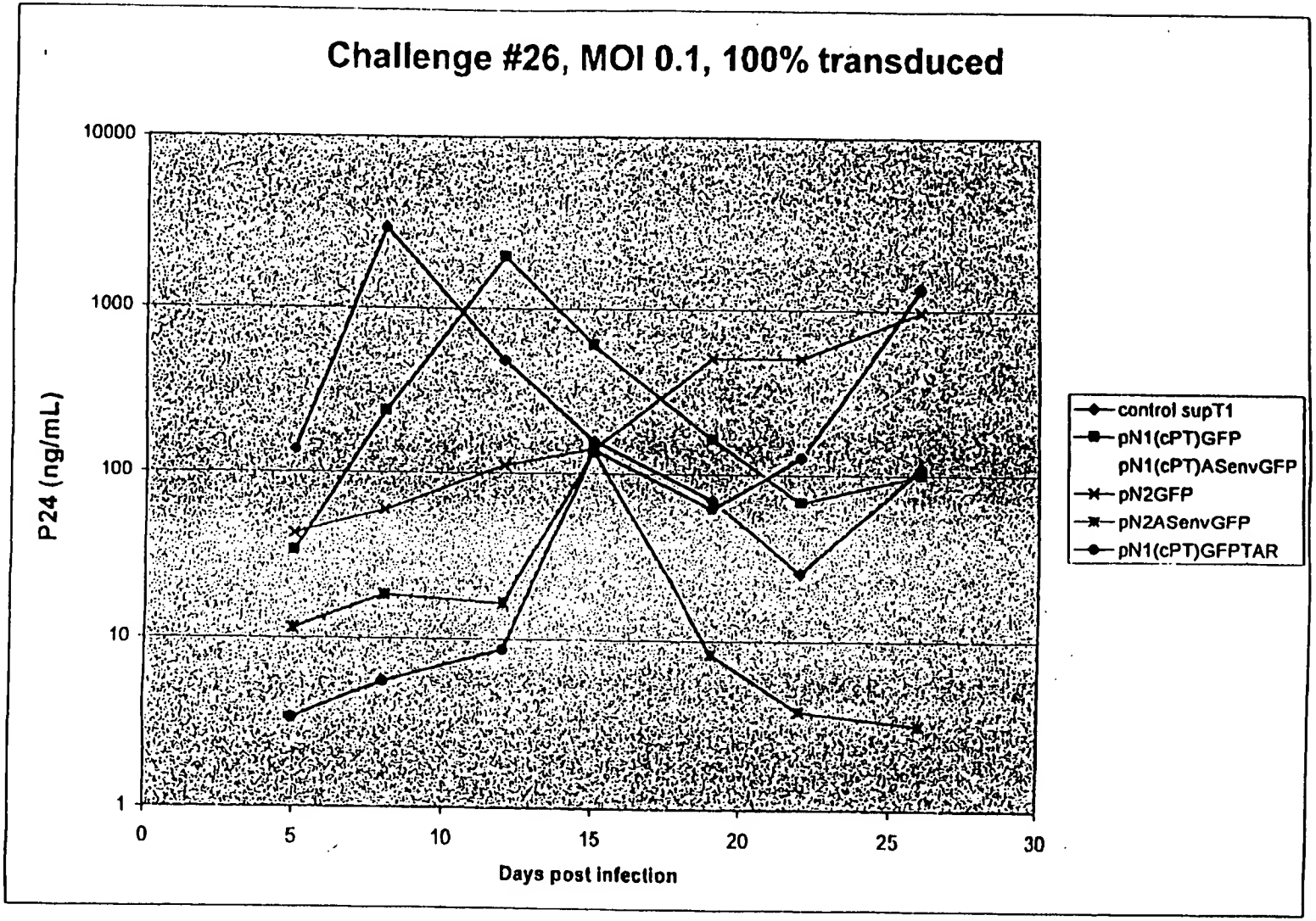


FIGURE 9A

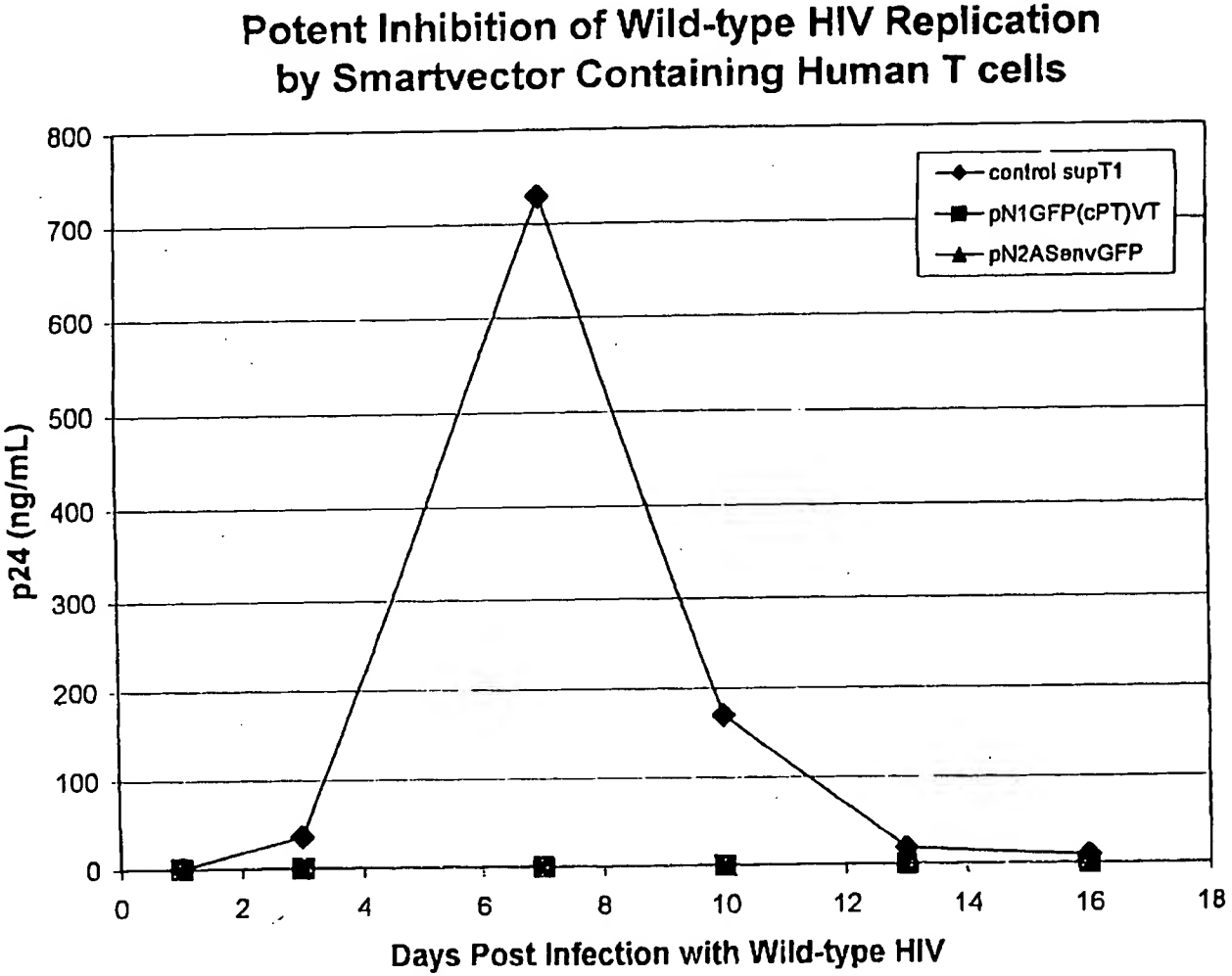


FIGURE 9B

Potent Inhibition of Wild-type HIV Replication by Smartvector Containing T Cells

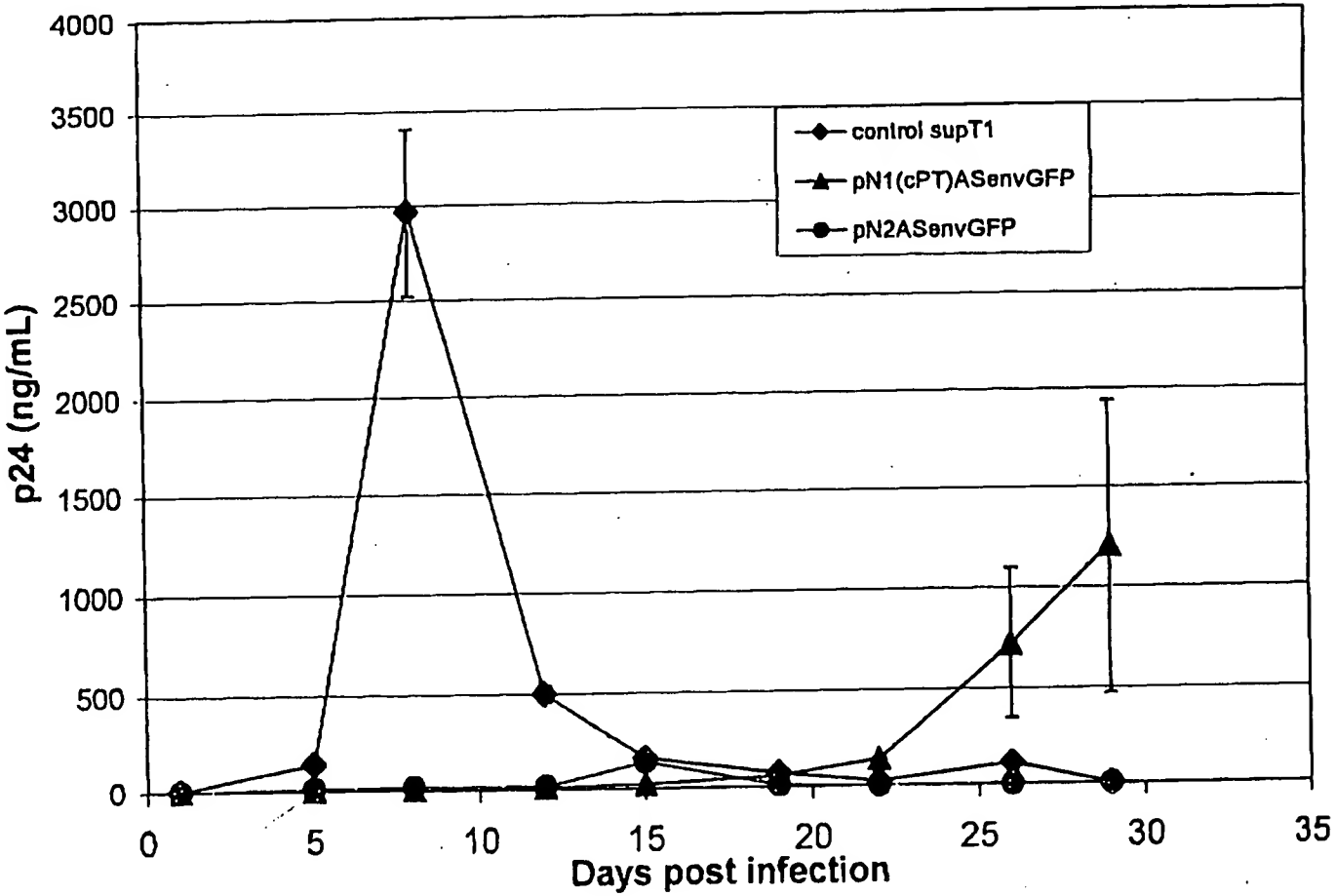
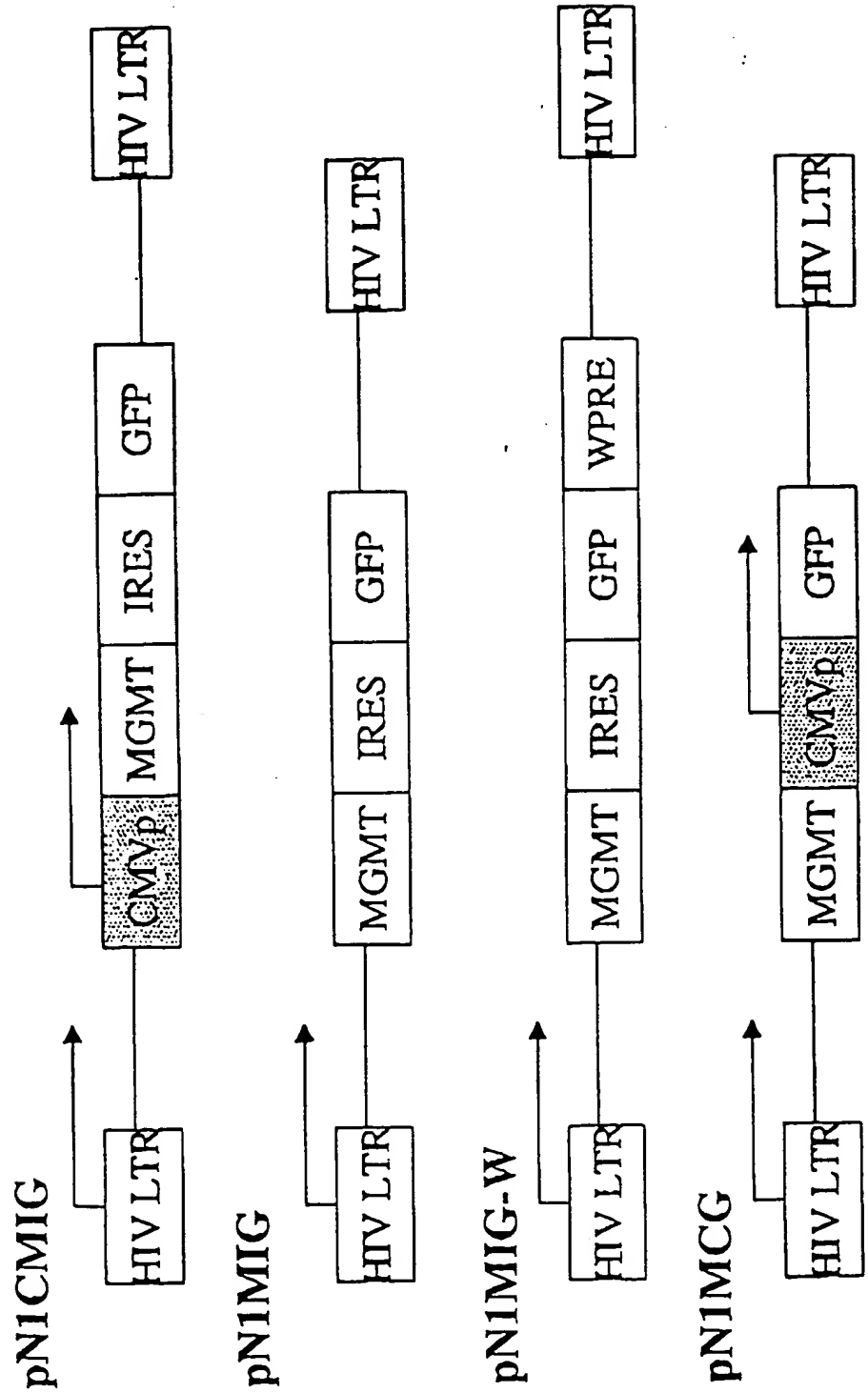
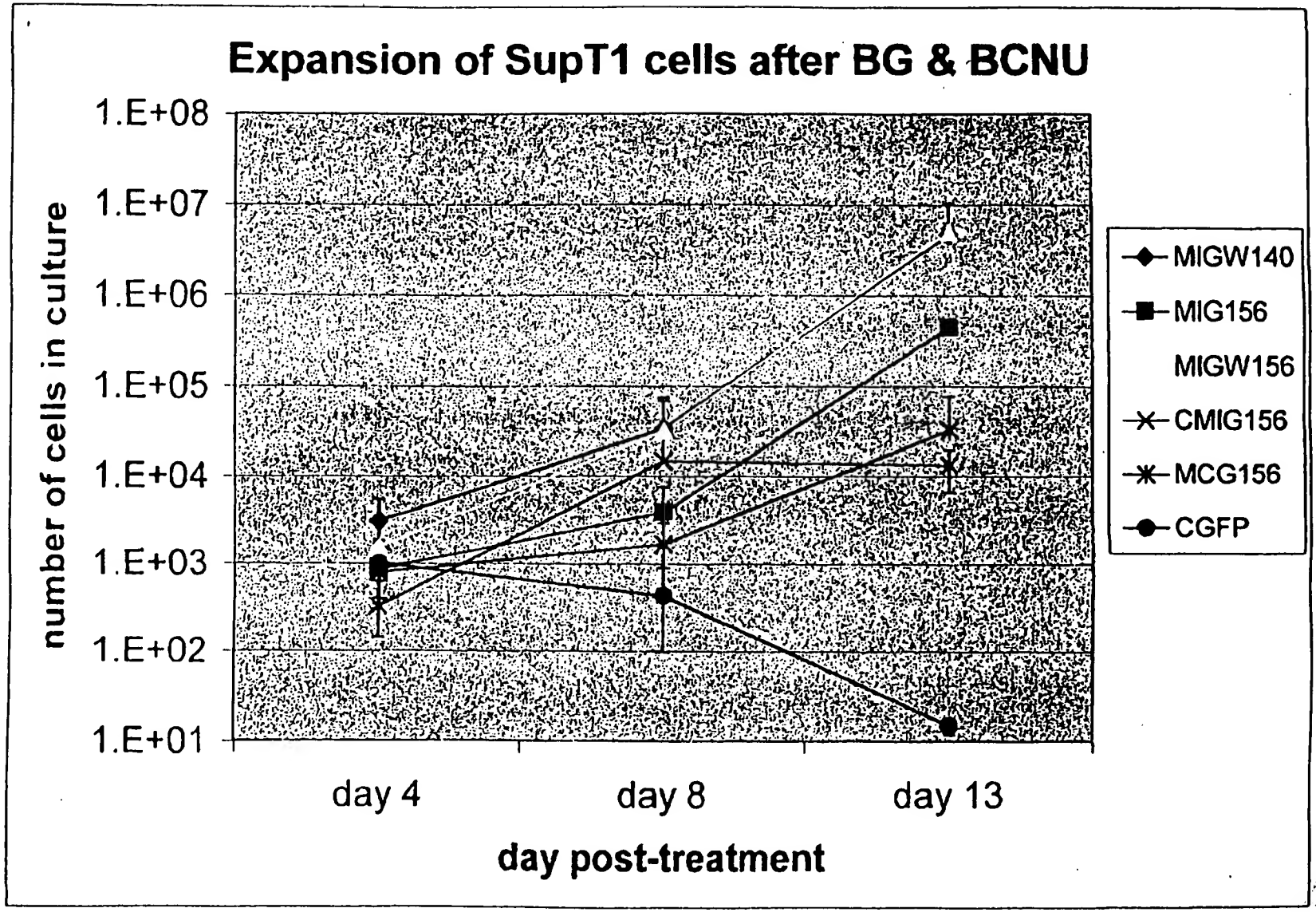


Fig 10A


pN1CMIG
HIV LTR
CMVp
MGMT
IRES
GFP
HIV LTR
pN1MIG
HIV LTR
MGMT
IRES
GFP
HIV LTR
pN1MIG-W
HIV LTR
MGMT
IRES
GFP
WPRE
HIV LTR
pN1MCG
HIV LTR
MGMT
CMVp
GFP
HIV LTR



Title: IMPROVED CONDITIONALLY REPLICATING VECTORS FOR INHIBITING VIRAL INFECTIONS
Inventor: Laurent HUMEAU et al
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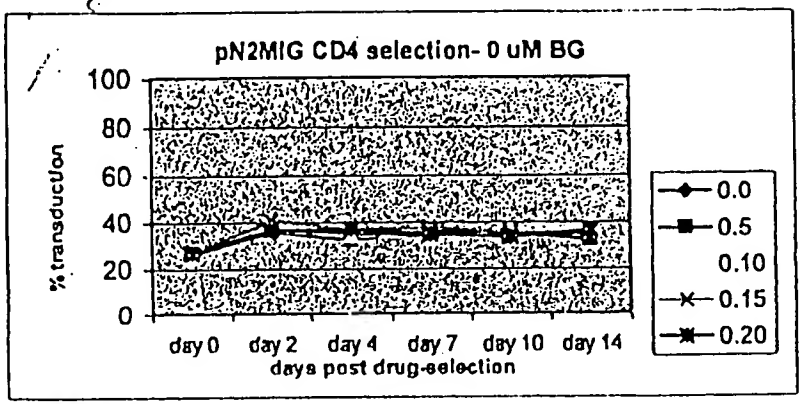
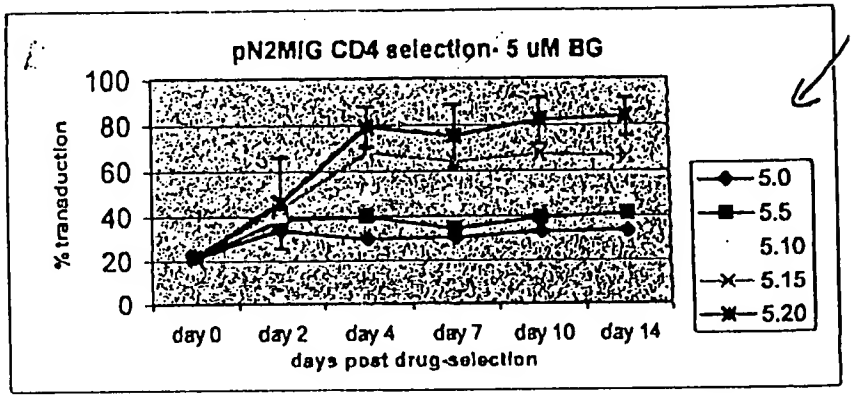
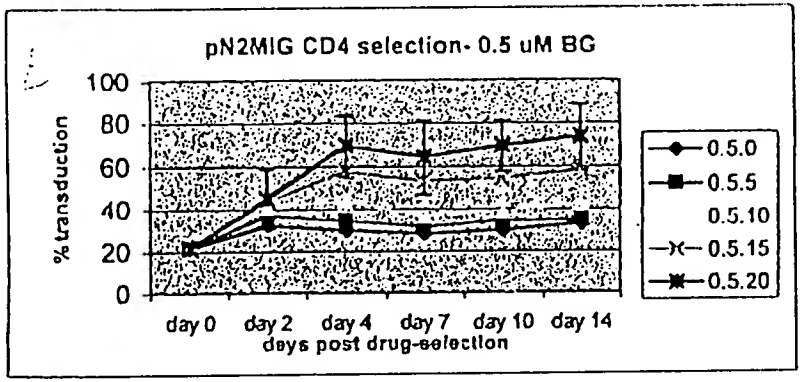
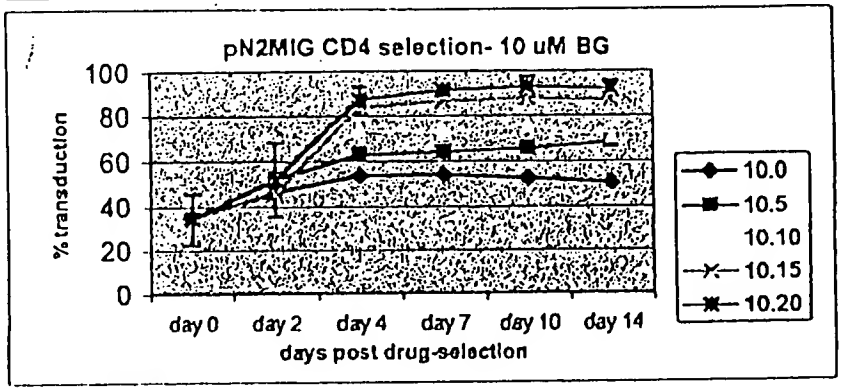
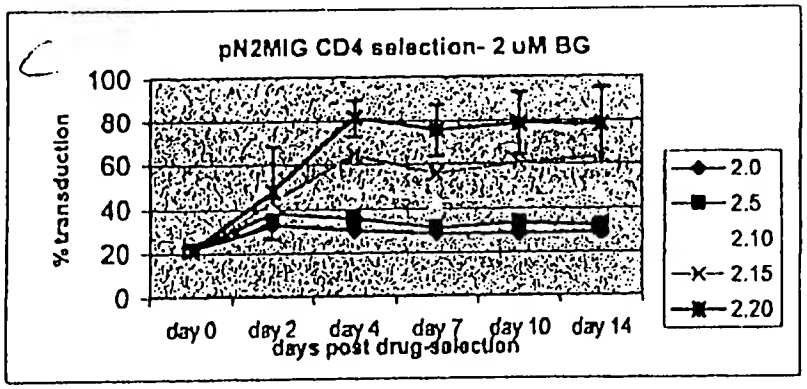
Fig 11

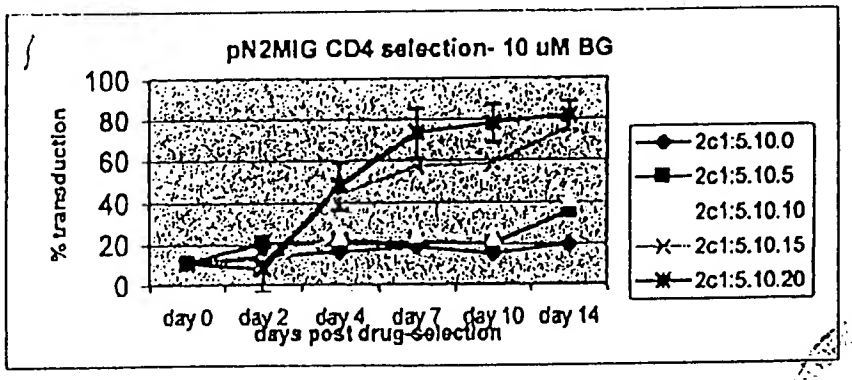












Title: IMPROVED CONDITIONALLY REPLICATING VECTORS FOR INHIBITING VIRAL INFECTIONS
Inventor: Laurent HUMEAU et al
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Fig 12

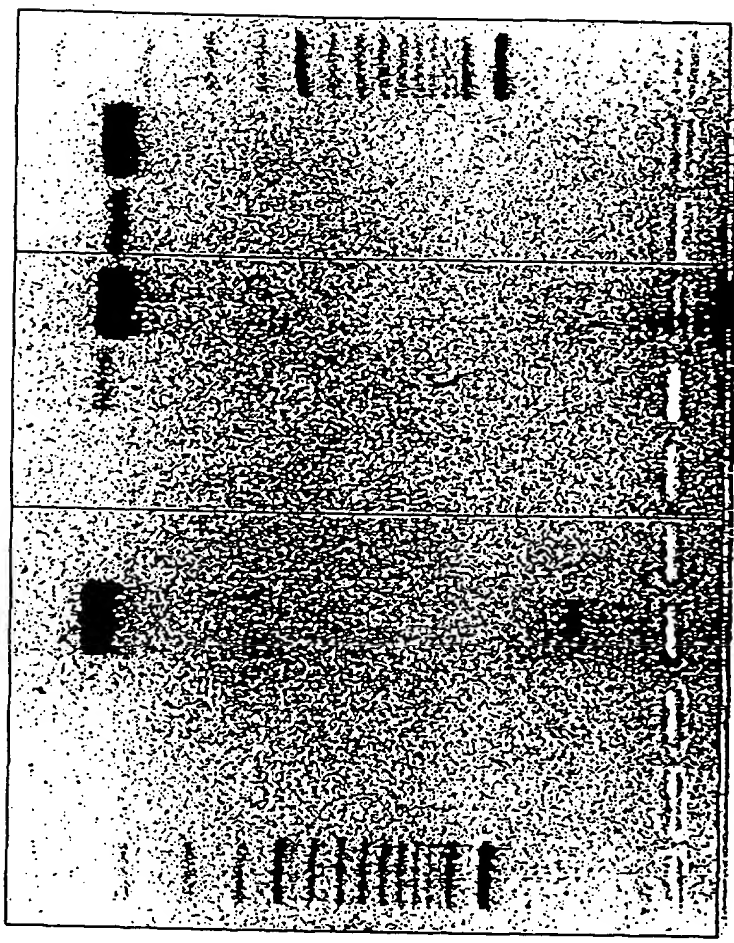
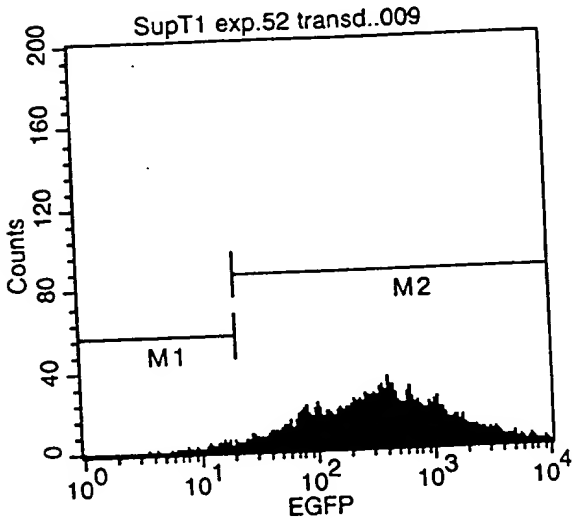


Fig 13A



Histogram Statistics

File: SupT1 exp.52 transd..009
Tube: pN1(cPT)ASenvGFP 452 a

Sample ID: SupT1 e›
Acquisition Date: 25-

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	6356	100.00	63.56	570.39
M1	1, 20	95	1.49	0.95	13.86
M2	20, 9910	6262	98.52	62.62	578.74

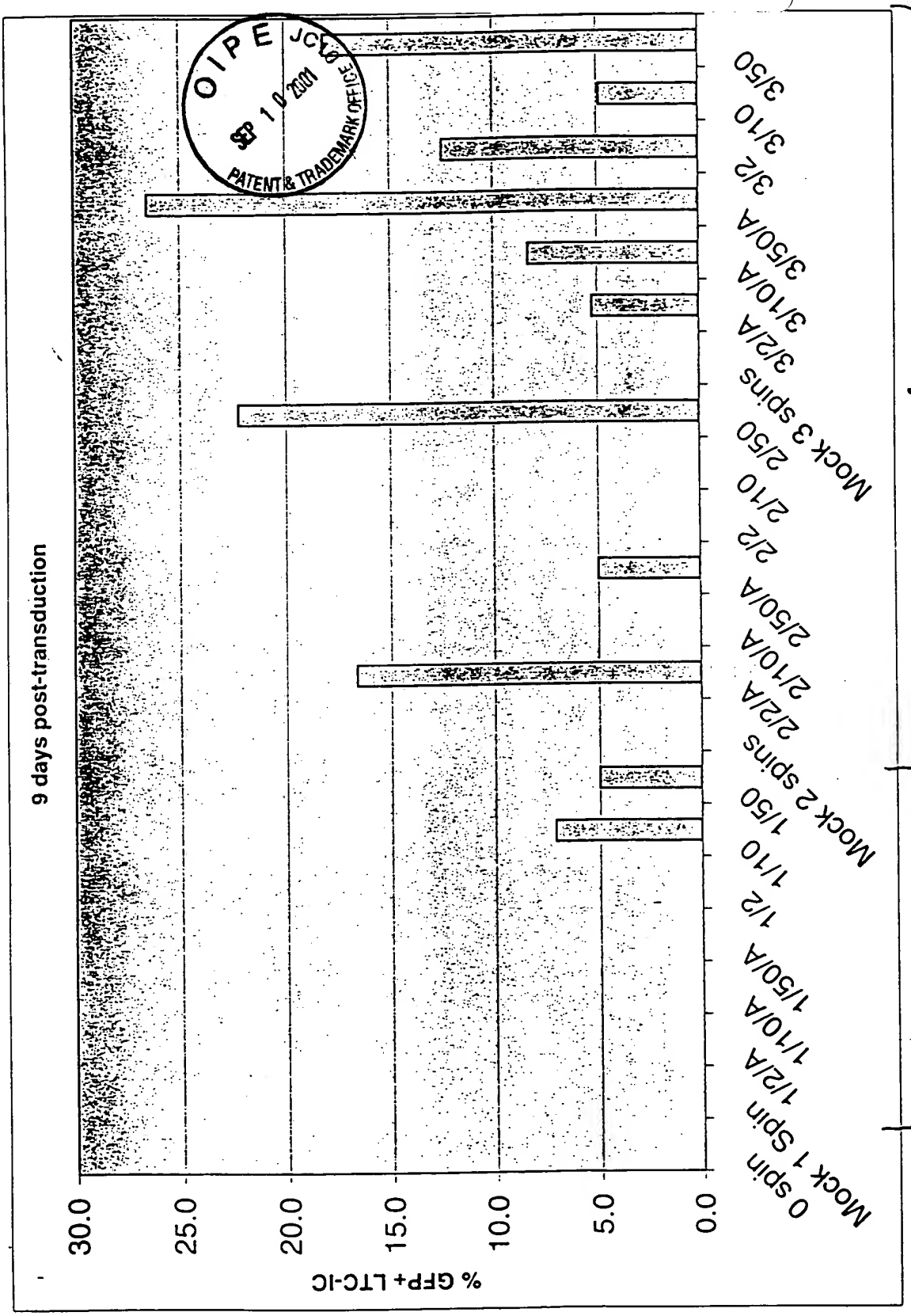


Fig 13B

Fig 14A

Vsv-G, RD114 AND RD114-VSV-G CHIMERIA ENVELOPE PROTEINS

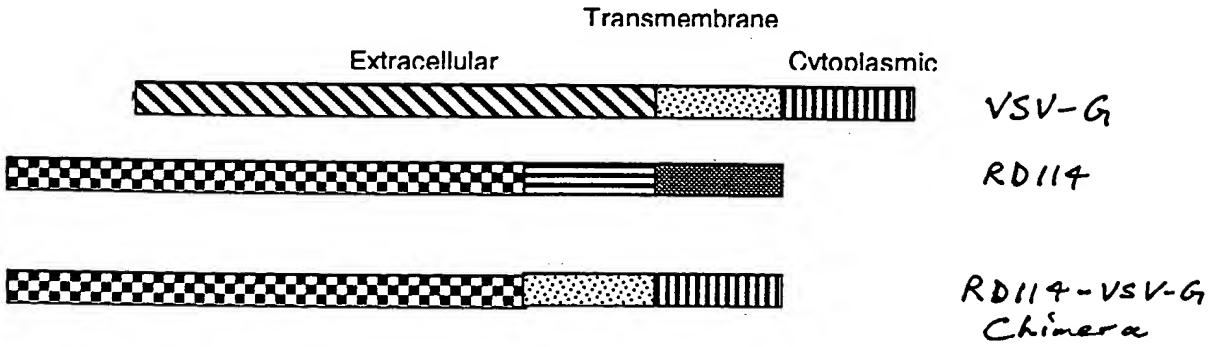


Fig 14B

Titers of RD114-pseudotyped HIV-1 vectors in HT1080

Envelopes	IU/ml
VSV G	3.5x10e6
Rabies virus G	1.6x10e6
RD114WT env	1.5x10e5
RD114E env	3.8x10e4

Fig 15A

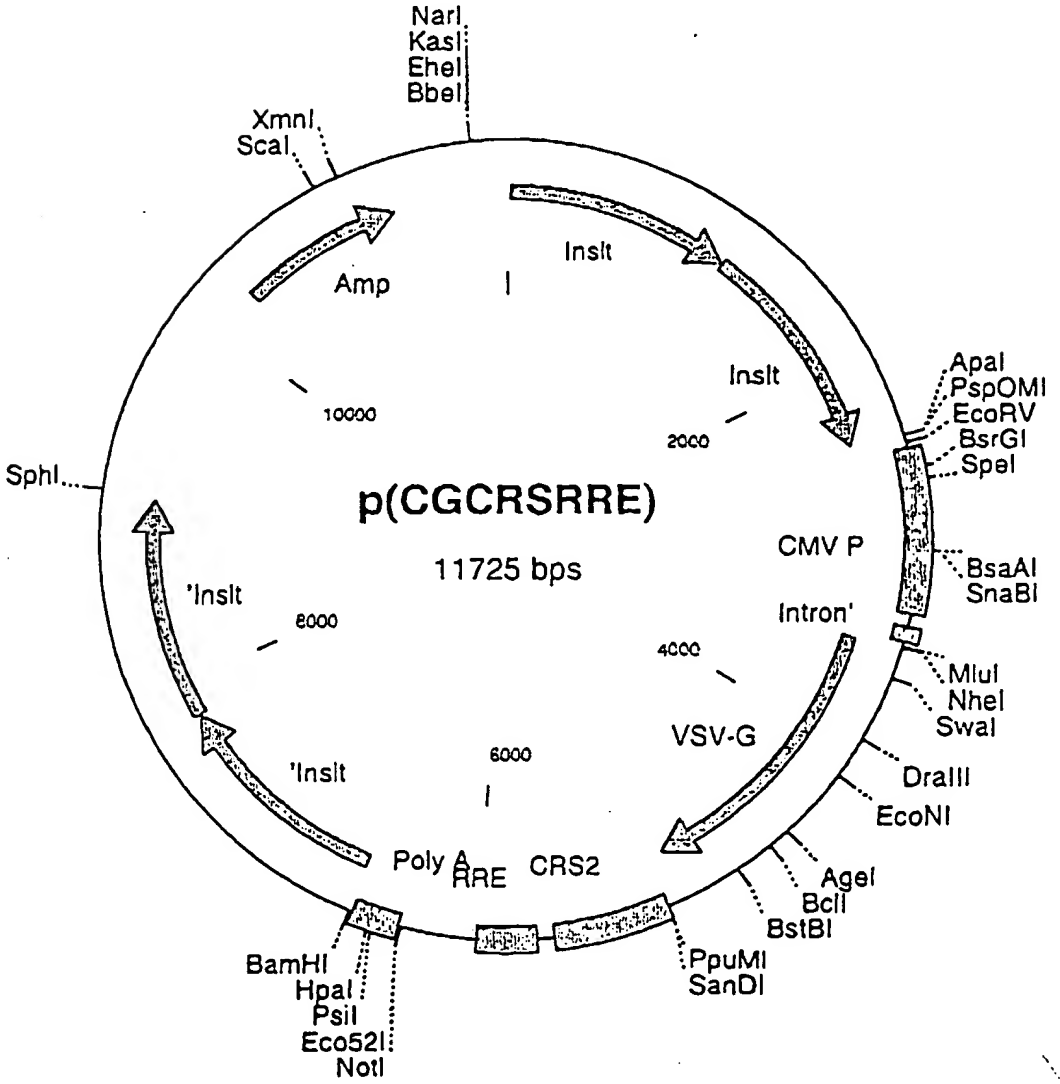

NarI
KasI
EheI
BbeI
XmnI
ScaI
InsIt
Amp
InsIt
ApaI
PspOMI
EcoRV
BsrGI
SpeI
SphI
10000
2000
p(CGCRSRRE)
11725 bps
CMV P
BsaAI
SnaBI
'InsIt
8000
Intron'
4000
MluI
NheI
SwaI
VSV-G
6000
'InsIt
DraIII
EcoNI
Poly A
RRE
CRS2
AgeI
BclI
BstBI
PpuMI
SanDI
BamHI
HpaI
PsiI
Eco52I
NotI

Fig 15A

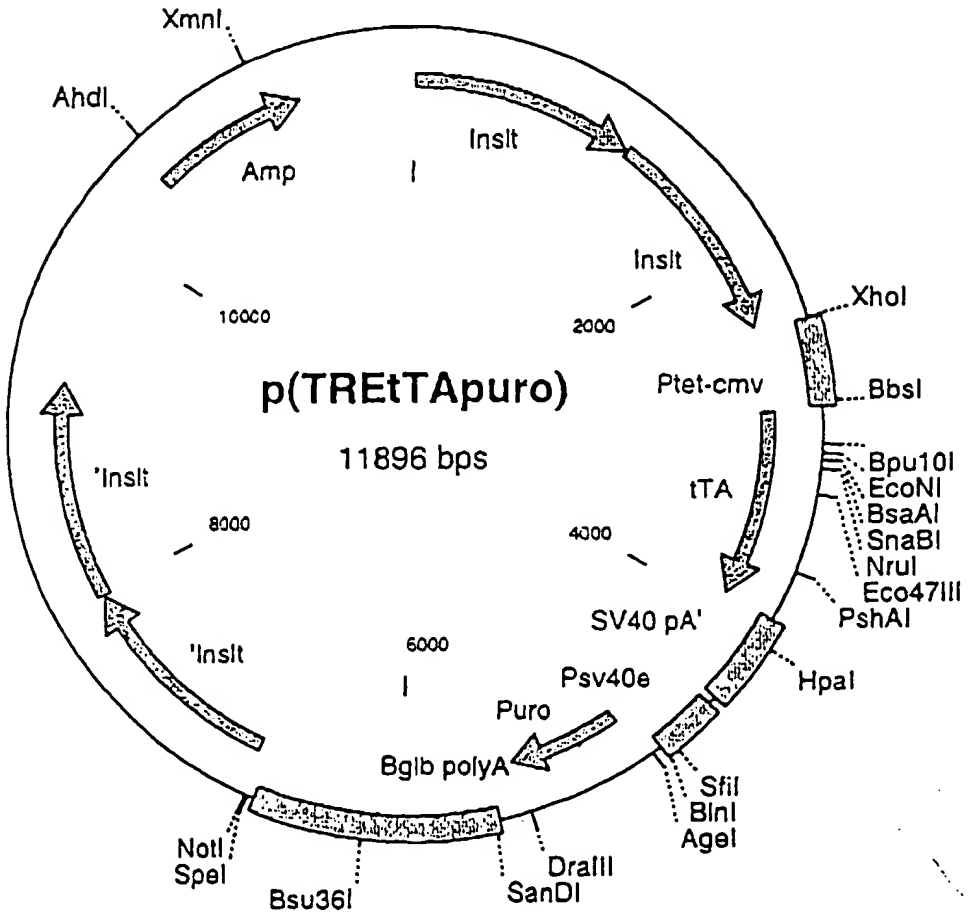


Fig 15C

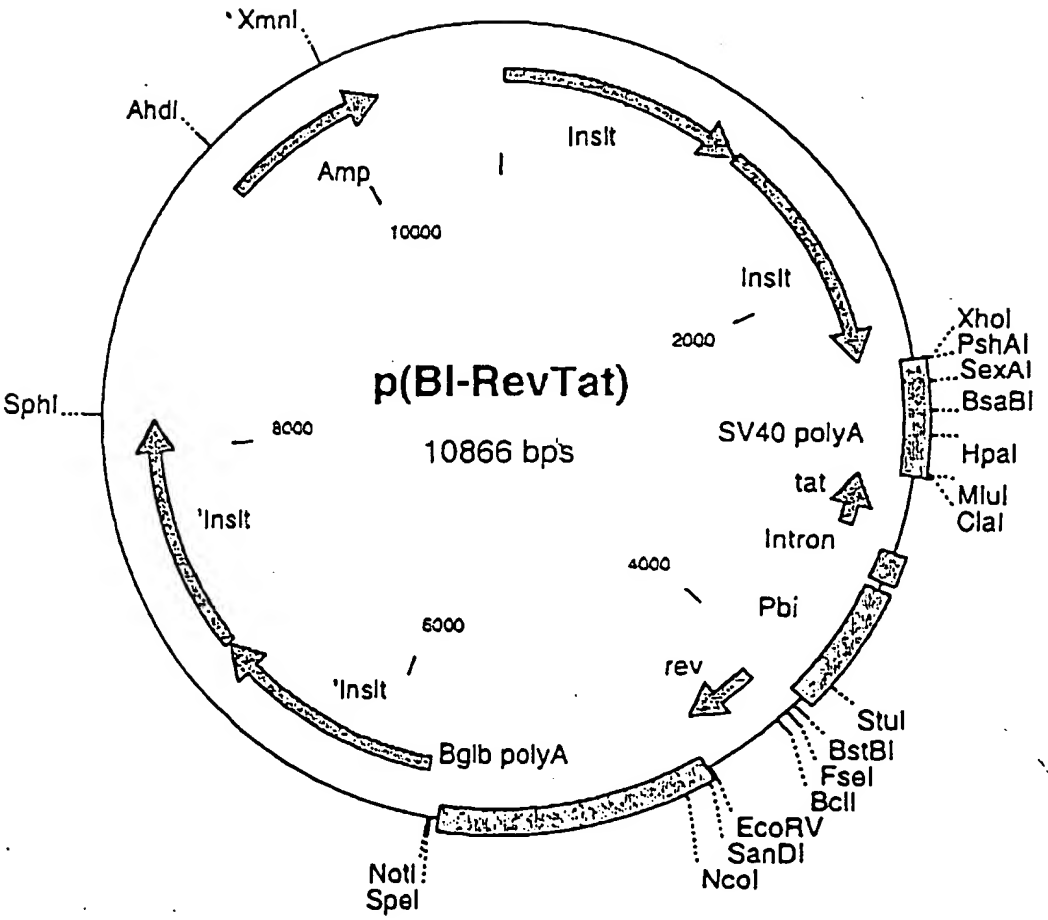

XmnI
AhdI
Amp
10000
InsIt
InsIt
2000
p(Bl-RevTat)
10866 bp's
SV40 polyA
tat
Intron
4000
Pbi
rev
6000
'InsIt
'InsIt
SphI
8000
BglB polyA
NotI
SpeI
EcoRV
SanDI
NcoI
StuI
BstBI
FseI
BclI
XhoI
PshAI
SexAI
BsaBI
HpaI
MluI
ClaI

Fig 15D

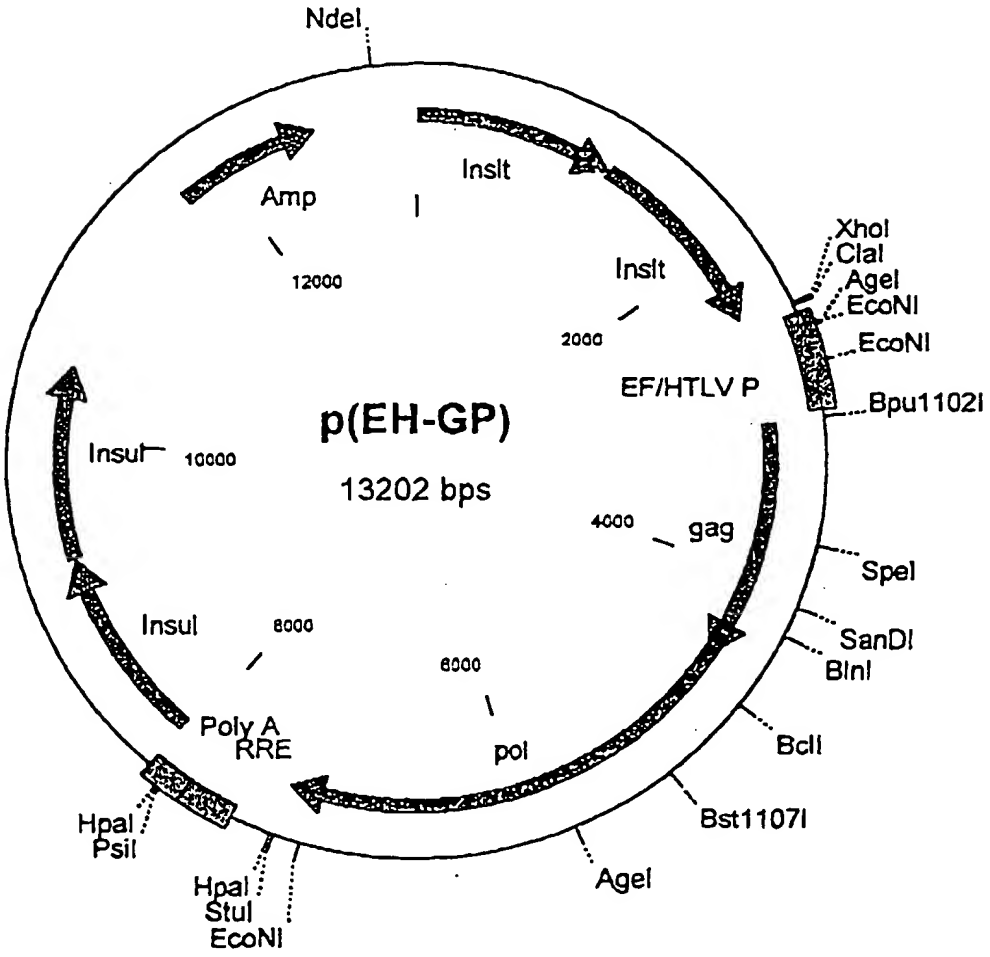

Ndel
InsIt
Amp
12000
InsIt
2000
XhoI
ClaI
AgeI
EcoNI
EcoNI
Bpu1102I
EF/HTLV P
p(EH-GP)
13202 bps
Insul
10000
4000
gag
SpeI
SanDI
BlnI
Insul
8000
6000
pol
BclI
Poly A
RRE
Bst1107I
HpaI
PsiI
AgeI
HpaI
StuI
EcoNI

Fig 15E

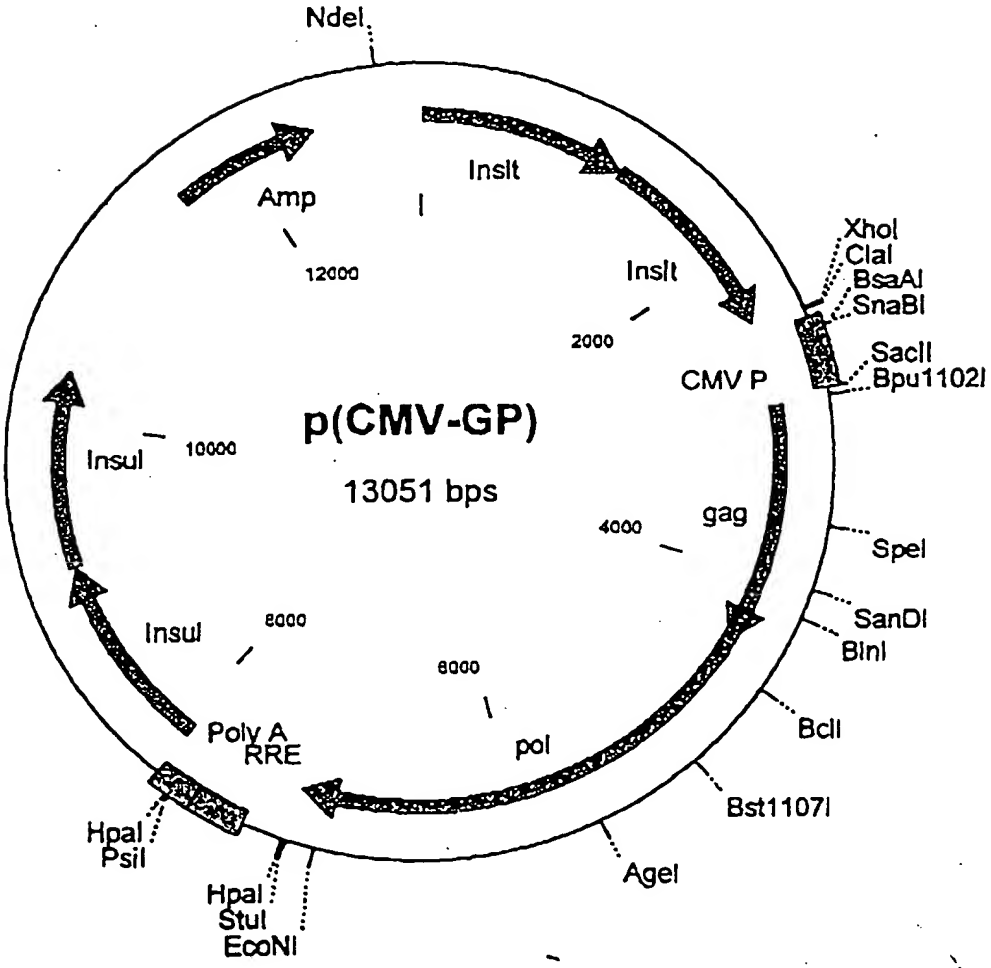

NdeI
InsIt
Amp
12000
InsIt
2000
XhoI
ClaI
BsaAI
SnaBI
SacII
Bpu1102I
CMV P
p(CMV-GP)
13051 bps
10000
InsuI
4000
gag
SpeI
SanDI
BlnI
InsuI
8000
6000
BclI
Poly A
RRE
pol
Bst1107I
HpaI
PsiI
AgeI
HpaI
StuI
EcoNI

Fig 15F

Rev dependent VSV-G constructs

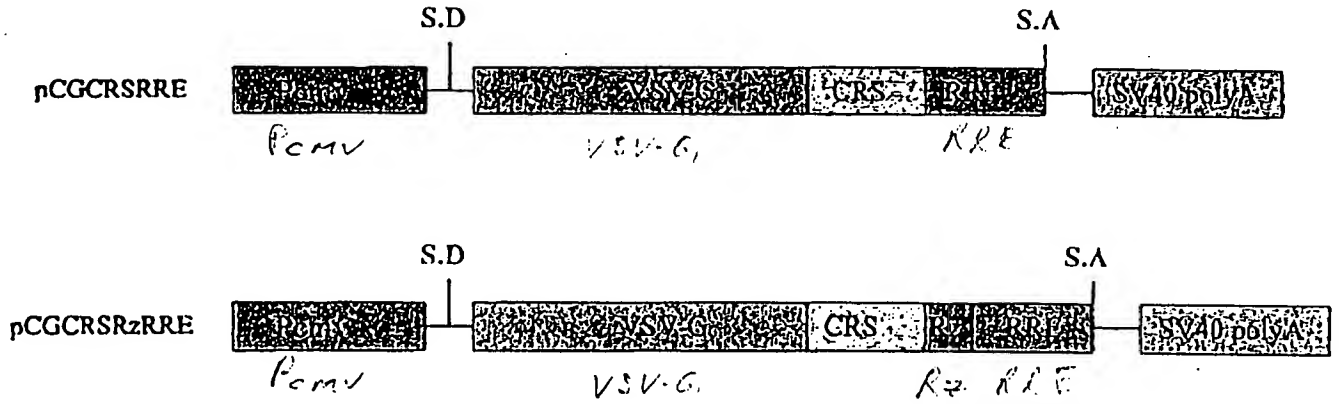


Figure 2

Yeild of pN1(cPT)GFP Vectors per Cell Factory before and after Concentration in HeLa-tat Cells.

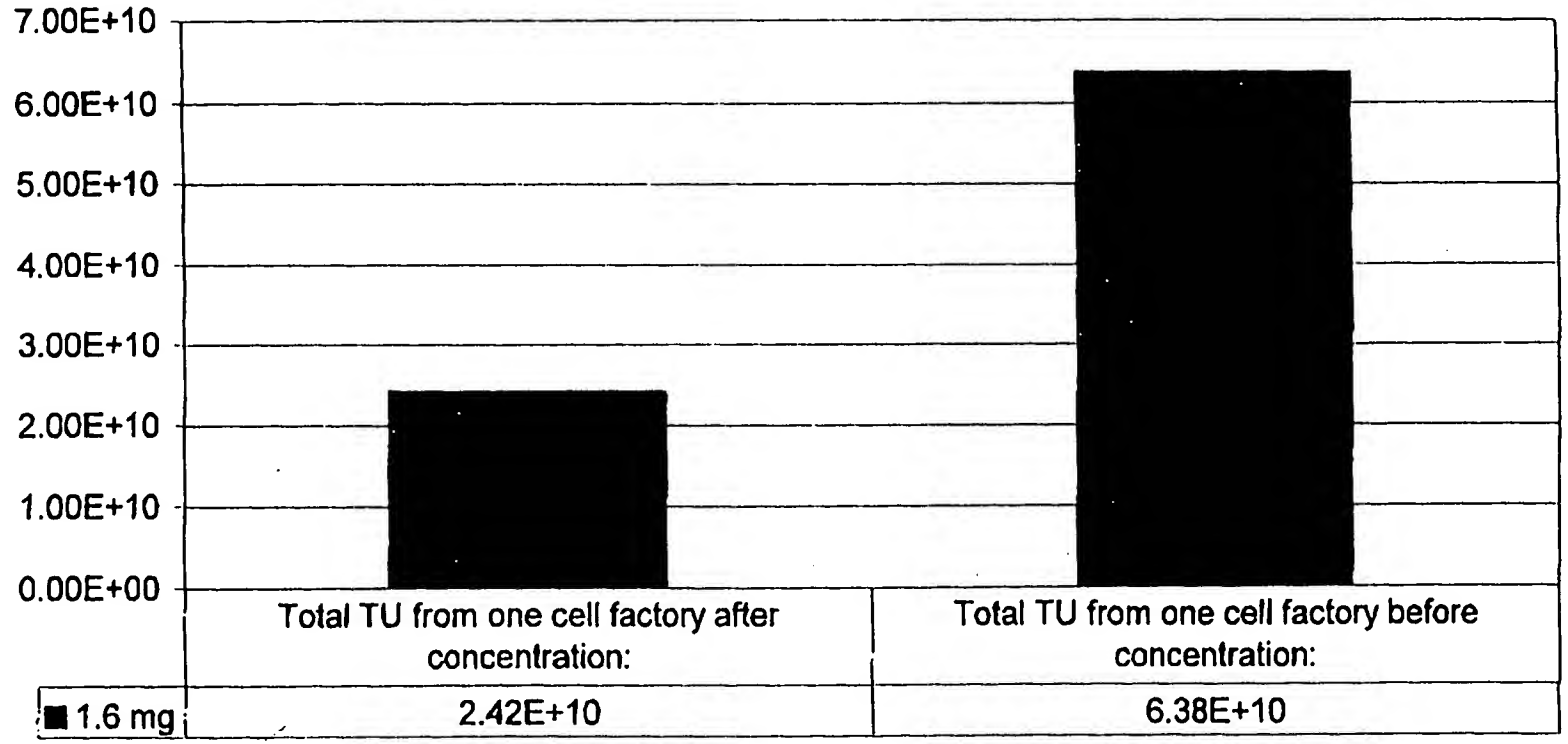
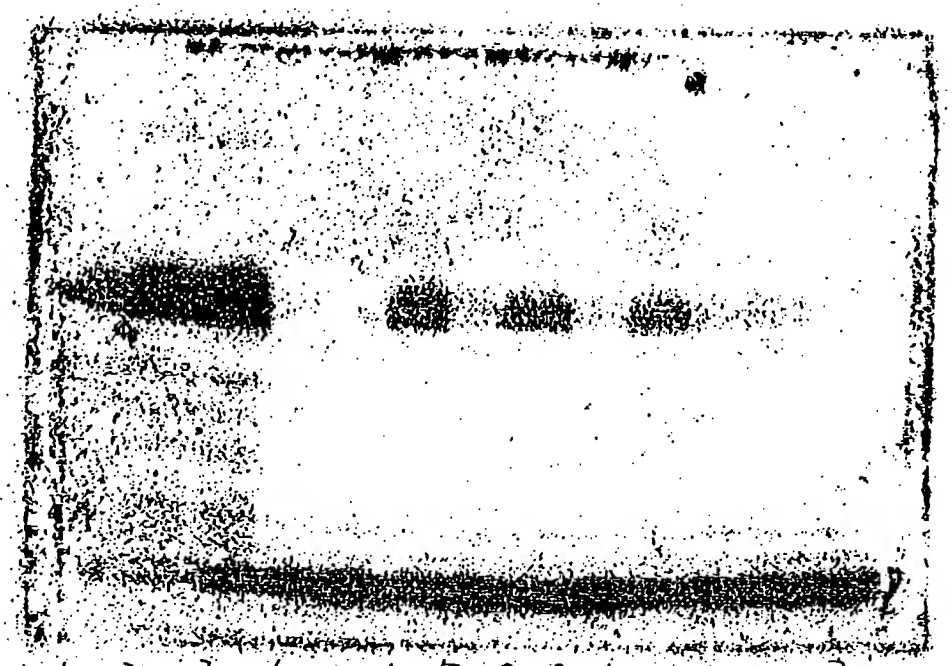


Fig 16

AFTER CONCENTRATION

BEFORE CONCENTRATION.

Fig 17



+ : pCMV-Rev

− : pCI

G : β-globin SD

1M — HIV-1 major SD

H — Hammarskjold's SD Analog

1E — HIV-1 env SD

2E — HIV-2 env SD

REMOVE TETRACYCLINE TO INDUCE EXPRESSION OF VSV-G THAT IS REV DEPENDENT.

Fig 18

Influence of the Buffer on Vector Recovery after Storage for 3-5 Weeks at Different Temperatures

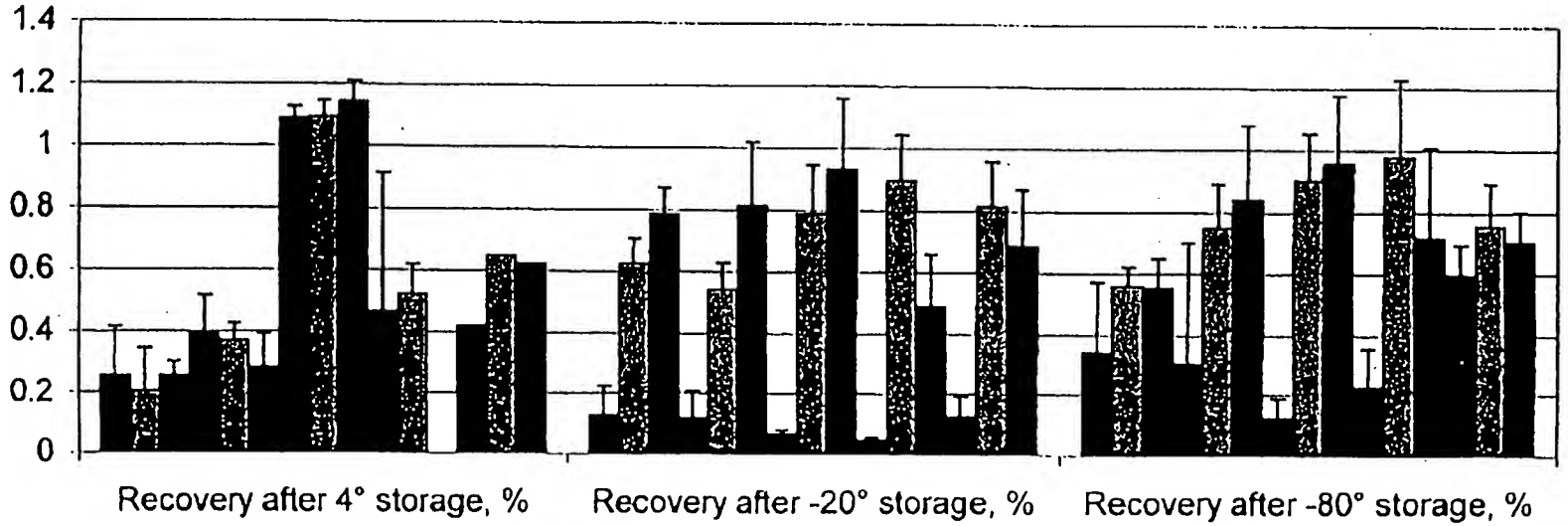


Figure 19